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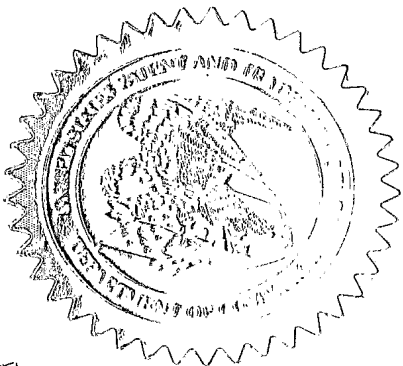
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**APPLICATION NUMBER: 60/548,401**

**FILING DATE: March 01, 2004**

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13281 U.S. PTO

**PROVISIONAL APPLICATION FOR PATENT COVER SHEET**This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 CFR 1.53 (b)(2).

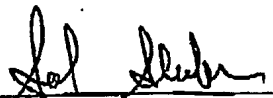
Docket Number		<b>27567</b>		Type a plus sign (+) inside this box ->	+
<b>INVENTOR(s) / APPLICANT(s)</b>					
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)		
SIDELMAN	Zvi		Tel Aviv, Israel		
TITLE OF THE INVENTION (280 characters max)					
CASEIN DERIVED PEPTIDES AND THERAPEUTIC USES THEREOF					
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STATE	VIRGINIA	ZIP CODE	22202	COUNTRY	USA
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification	Number of Pages	155	<input checked="" type="checkbox"/> Applicant is entitled to Small Entity Status		
<input checked="" type="checkbox"/> Drawing(s)	Number of Sheets	30	<input checked="" type="checkbox"/> Other (specify) Assignment		
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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees			FILING FEE AMOUNT (\$)		\$ 80.-
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No☐ Yes, the name of the US Government agency and the Government contract number are: \_\_\_\_\_

Respectfully submitted,

SIGNATURE



29 February 2004

Date

25,457

REGISTRATION NO.  
(if appropriate)TYPED or PRINTED NAME SOL SHEINBEIN☐ Additional inventors are being named on separately numbered sheets attached hereto**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

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**CASEIN DERIVED PEPTIDES AND THERAPEUTIC USES THEREOF****FIELD OF THE INVENTION:**

The present invention relates to biologically active peptides that are  
5 derived from or are similar to sequences of the  $\alpha$ S1-,  $\alpha$ S2-,  $\beta$ - or  $\kappa$ -casein  
fractions of milk casein. These peptides are capable of immune modulation and  
other therapeutic activities, including but not limited to stimulating and  
enhancing immune response, protecting against viral infection, normalizing  
serum cholesterol levels, and stimulating hematopoiesis. The casein-derived  
10 peptides are non-toxic and can be used to treat and prevent immune  
pathologies, diabetes, hypercholesterolemia, hematological disorders and viral-  
related diseases.

**BACKGROUND OF THE INVENTION*****Bioactive molecules from nutrients:***

In addition to the nutritional value of many foods, certain fractions and  
products of digestive pathways possess the ability to influence physiological  
processes. Some of these "extranutritional" constituents are present in their  
active form in the whole nutriment, such as the immunoglobulins in mother's  
20 milk and colostrums, phytoestrogens found in soy-based foods, polyphenolic  
antioxidants from fruits and vitamins. Others are encrypted within nutrient  
molecules, and are released in an active form during digestion or food  
processing, for example antihypertensive peptides from lactoglobulin [Kitts, D.  
D. (1999), Can. J. Physiol. Pharmacol. 72:4; 423-434].

***Biological activity in milk proteins:***

Milk contains a wide variety of proteins that contribute to it's unique  
qualities. Some proteins, such as bile-salt stimulated lipase, amylase, beta-  
casein, lactoferrin, haptocorrin and alpha-antitrypsin assist in digestion and  
utilization of milk-derived nutrients. Other proteins, such as immunoglobulins,  
30 kappa-casein, lysozyme, lactoferrin and lactalbumin may, in the intact or



partially digested form, have immunomodulatory and antimicrobial activity. Casein, the predominant milk protein, has been traditionally defined as composed of three fractions,  $\alpha$ ,  $\beta$  and  $\gamma$ , according to their electrophoretic mobility [N. J. Hipp, *et al.* (1952), Dairy Sci., 35:272]. Today casein is defined  
5 according to the amino acid sequences of each of the subgroups  $\alpha$ S1,  $\alpha$ S2,  $\beta$  and  $\kappa$  [W. N. Engel *et al.* (1984), J. Dairy Sci. 67: 1599].

In the course of digestion, the casein proteins are subjected to proteolytic cleavage by acid proteases such as chymosin (rennin), trypsin and pepsin, producing shorter peptides and causing curdling and calcium sequestration by  
10 the resultant protein fragments. A few studies with milk compounds demonstrated casein-related bacteriocidal activity. U.S. Patent No. 3,764,670 discloses proteolytic casein digests possessing antibiotic properties against microorganisms. Israel Patent No. 42863 describes a casein-derived peptide consisting of 23 amino acids of the N-terminus of casein, possessing anti-  
15 bacterial activity. Shimizu *et al.* describe a short N-terminal fragment derived from  $\alpha$ S1 casein peptic hydrolyzate having emulsifying properties, suggesting that this might be somehow useful to the food industry (Shimizu, *et al.* J of Food Science, 1984;49: 1117-20). The authors investigated the amino acid composition of the fragment, its in-vitro emulsifying activity, and noted that it  
20 resembled a 23 amino acid long N-terminal fragment of  $\alpha$ S-1, concluding that the fragments were identical. However, no proof of identity was provided, and no biological activity was investigated.

In another study, Chabance *et al.* (Biochimie 1998;80:155-65) detected the presence of casein-derived peptides and peptide fragments in the stomachs  
25 and blood of humans after ingestion of yoghurt and milk. The authors reported the presence of fragments of bioactive  $\kappa$ -casein (caseinoglycopeptide) and an N-terminal fragment of  $\alpha$ S-1 casein having antibacterial activity, in the blood following digestion. They concluded that the passage of these peptides,

unaltered, into the plasma suggests a common, transport pathway for their duodenal absorption. No activity of the peptide fragments was demonstrated.

Lahov and Regelson describe a brief (30 minutes) chymosin digest of whole, acid-precipitated bovine and human casein, to yield a fraction enriched in an  $\alpha$ S-1 casein N-terminal peptide (Lahov and Regelson, *Fd Chem Toxic* 5 1996;34:131-45), essentially duplicating the teachings of U.S. Patent No. 3,764,670 to Katzir-Katchalsky et al. The chymosin digest was then precipitated with TCA, and characterized by centrifugal analysis and short column equilibrium methods. The authors report an N-terminal  $\alpha$ S-1 casein 10 peptide fragment, similar to the anti-bacterial "isracidin" reported by Katzir-Katchalsky et al. However, the veracity of the author's claims to purification to homogeneity are questionable, considering the repeated detection of mixture of peptides reported in detailed studies of chymosin digest of casein employing sensitive analytical techniques (see, for example, Carles et al, *FEBS Lett.* 15 1985;115:282-6; McSweeney et al, *J Dairy Res.*,1993;60:401-12, and Yvon, et al.*Int. J. Pept. Prot Res*, 1989;34:166-76).

In addition, other physiologically active properties, such as opioid and growth factor-like activities have been proposed for casein or its derivatives [Kitts, D. D., (1999), *ibid.*].

20 Immune modulating activity has also been observed in casein peptides. Coste et al.[Coste et al. (1992), *Immun. Lett.* 33: 41-46)] observed enhancement of rat lymphocyte proliferation following treatment with a peptide derived from the C-terminus of  $\beta$  casein. U.S. Patent Nos. 5,506,209, 5,538,952 and 5,707,968, all to Mukerji et al, teach the administration of human 25  $\beta$ -casein, recombinant human  $\beta$ -casein, and hydrolysates of both, in a liquid enteral formula, for treating respiratory syncytial virus, otitis media, H. influenza and other infections in infants. Bovine  $\beta$ -casein was tested, but found to lack significant inhibitory activity, leading the authors to conclude that " $\beta$ -casein from human milk has different bioactivity compared to bovine  $\beta$ -casein".

U.S. Patents 5,147,853 and 5,344,820 to Dosaka, et al. teach the administration of a sialic-acid conjugated  $\kappa$ -casein and  $\kappa$ -casein-derived glycomacropptide (GMP) from cow's milk for prevention of bacterial and viral infections in vitro and in vivo in rats. U.S. Patent No 5,330,975 to Isoda, et al. teaches the use of sialic-acid binding  $\kappa$ -casein and  $\kappa$ -casein peptides for the neutralization of bacterial endotoxins, such as cholera toxin. Similarly, U.S. Patent Nos. 5,712,250 to Mukerji, et al, and 5,968,901 to Andersson, et al, teach the use of human  $\kappa$ -casein, but not bovine  $\kappa$ -casein, for the prevention of bacterial and H. influenza infection. However, these casein compositions taught in the prior art are relatively crude, even following gross fractionation, and none of these studies have determined the specific sequences in these casein peptides which confer their "extranutritional" properties.

Recent studies have detected a correlation between the consumption of the A1  $\beta$ -casein fraction of bovine milk and Ischemic Heart Disease (IHD) in many Western countries (see, for example, M. Laugesen, NZ Med J. 2003;116:U295), leading to development of A1  $\beta$ -casein-free milk (U.S. Patent No. 6,570,060 to McLachlan).

***Hematopoiesis in cancer therapy:***

Following high-dose chemotherapy, especially following myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT), patients are at high risk due to pancytopenia. Granulocytopenia may lead to development of serious, occasionally fatal infectious complications from common bacterial, viral, fungal and parasitic agents in the immediate post transplant period. Similarly, thrombocytopenia frequently results in bleeding tendency and occasionally, in long lasting platelet dependence. Whenever resistance to platelets develops, bleeding episodes can be life threatening and hemorrhagic complications are frequently lethal. The risk due to granulocytopenia can be partially overcome by supportive measures and most effectively by administration of recombinant human cytokines that

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can enhance reconstitution of granulocytes, particularly granulocyte colony stimulation factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF). These agents are extremely expensive (approximately \$200-400/day/patient) and infrequently cause side effects due to hypersensitivity reactions, fever, bone pain and occasionally vascular leak syndromes, including pericarditis and pleuritis. Some of the side effects may be due to other cytokines that may be intrinsically released by these hematopoietic growth factors. Moreover, the use of these hematopoietic growth factors may be prohibitive in patients with tumor cells bearing G-CSF or GM-CSF receptors such as in acute and chronic myeloid leukemias and in myelodysplastic syndromes. Whereas major progress in treating patients at risk of pancytopenia has been achieved from the use of hematopoietic cytokines, no progress has been made in the treatment of thrombocytopenia. Following high dose chemotherapy and especially following ASCT, patients are at risk for thrombocytopenia which may last for many months even up to 3 years and some thrombocytopenic patients may never recover. Many patients previously treated with multiple blood products become platelet resistant and hence thrombocytopenia may be impossible to overcome, even transiently, despite intensive and frequent platelet transfusions from a single donor. Resistance to platelets and protracted thrombocytopenia represent a common cause of death at ASCT centers worldwide.

Currently, several new recombinant cytokines such as recombinant human interleukin-3 (rhIL3) and recombinant human interleukin-6 (rhIL6) are being investigated as potential agents for enhancing megakaryocytopoiesis and platelet reconstitution. Unfortunately, preliminary clinical trials showed that although rhIL3 and rhIL6 may enhance platelet reconstitution, such effects are by no means dramatic and may take considerable time.

Clearly, protracted thrombocytopenia represents a major problem in clinical Bone Marrow Transplant centers today, for which no satisfactory solution has yet been found.

There is thus a widely recognized need for, and it would be highly advantageous to have a safe, inexpensive, rapidly effective and well-defined stimulator of hematopoiesis, and specifically megakaryocytopoiesis, devoid of the above limitations.

5        ***Thrombopoietin (TPO) in regulation of hematopoiesis and platelet function:***

TPO appears to be the major regulator of platelet production *in vivo*, although increase in the kidney- and liver-derived growth factor in platelet deficiencies is not caused by adaptation of TPO biosynthesis in these organs. Rather, a "feed-back loop" seems to exist in which the number of circulating platelets determines how much of the circulating TPO is available to the bone marrow for platelet production. In addition, it has been demonstrated that TPO is an early acting cytokine with important multilineage effects: TPO alone, or in combination with other early acting cytokines, can (i) promote viability and suppress apoptosis in progenitor cells; (ii) regulate hematopoietic stem cell production and function; (iii) trigger cell division of dormant multipotent cells; (iv) induce multilineage differentiation and (v) enhance formation of multilineage colonies containing granulocytes, erythrocytes, macrophages, and megakaryocytes (MK, CFU-GEMM). Moreover, TPO stimulates the production of more limited progenitors for granulocyte/monocyte, megakaryocyte and erythroid colonies, and stimulates adhesion of primitive human bone marrow and megakaryocytic cells to fibronectin and fibrinogen. Thus, TPO is an important cytokine for clinical hematologists/transplanters: for the mobilization, amplification and *ex vivo* expansion of stem cells and committed precursor cells for autologous and allogeneic transplantation [von dem Borne, A.E.G.Kr., et al., (1998) Thrombopoietin: it's role in platelet disorders and as a new drug in clinical medicine. In Bailliers Clin. Hematol. June:11(2), 427-45].

In addition to TPO effects in hematopoiesis, this potent growth factor primes platelets for various agonists and modulates platelet-extracellular matrix

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interactions. Although it does not itself cause platelet aggregation, TPO upregulates ADP-induced aggregation, especially on the second wave of aggregation, upregulates granule (ADP, ATP, serotonin, etc.) release and production of thromboxane B2, increases platelet attachment to collagen and potentiates shear-induced platelet aggregation. TPO also stimulates PMN  
5 activation, inducing IL-8 release and priming oxygen metabolite production, likely enhancing antimicrobial defense.

Clinical studies suggest TPO's value in understanding and treating a variety of hematological conditions. In patients with idiopathic aplastic anemia  
10 (AA), elevated TPO levels persist even in remission following immunosuppressive therapy, indicating a hematopoietic defect. TPO is elevated in other forms of aplastic thrombocytopenia as well, but not in conditions of increased platelet destruction. Apparently, the reactive increase in TPO production is insufficient in cases of destructive thrombocytopenia. Thus, TPO  
15 is not only a therapeutic option for aplastic, but also for destructive thrombocytopenia.

Thrombopoietic agents are of great clinical interest, for prevention and/or treatment of pathological or treatment-induced thrombocytopenia, and as a substitute for platelet transfusions. Of the cytokines evaluated, all but the  
20 marginally potent IL-11 have been deemed unacceptable for clinical use. TPO is widely believed to become the cytokine of choice for thrombocytopenia treatment. Recombinant human TPO (Genentech) has recently become available, enabling accurate pharmacokinetic determinations and clinical trials. Thus, TPO's potential applications encompass the realms of supportive care  
25 (post chemo/radio-therapy, bone marrow and stem cell transplantation), hematological disease (AA, myelodysplasia, congenital and acquired thrombocytopenia), liver diseases, transfusion (expansion, harvest, mobilization and storage of platelets) and surgery (including liver, transplantation). Of particular interest is the potential use of TPO/EPO/G-CSF cocktail for  
30 myelodysplasia, G-CSF and TPO combination for peripheral stem cell

mobilization and TPO in harvesting CD 34+ cells and *ex vivo* expansion of megakaryocytes for superior platelet reconstitution. Recombinant human G-CSF is also available (Filgrastim, Amgen, Inc. USA). However, similar to other hematopoietic agents under consideration for clinical application, TPO and G-CSF are costly and potentially antigenic at therapeutically effective levels. Thus, it would be advantageous to have a safe, inexpensive and readily available stimulator of thrombopoiesis and granulocytopoiesis capable of augmenting TPO and G-CSF activity.

**SARS:**

The worldwide outbreak of severe acute respiratory syndrome (SARS), and reported SARS-related deaths in more than 25 countries in the spring of 2003 have focused attention on the suspected infective agent, the SARS-CoV coronavirus (Rota et al, Scienceexpress 1 May 2003). Evidence of SARS-CoV infection has been documented in SARS patients throughout the world, SARS-CoV infection has been detected in respiratory specimens, and convalescent-phase serum from SARS patients contains anti-SARS antibodies. Presently, no therapies have been identified for the prevention or treatment of SARS-CoV infection.

In the absence of effective vaccines or drugs, the current SARS epidemic threatens to reach devastating proportions, similar to epidemics of other infectious diseases spread by respiratory route such as the influenza epidemic of 1918 and measles epidemics. As has been emphatically stated by many health officials, the key to controlling epidemics is the blockage of transmission of infection. Thus, in addition to much needed public health measures, the development of methods for prevention and/or treatment of SARS is of foremost importance.

**The  $\alpha$ ,  $\kappa$ -, and  $\beta$ - fractions of casein:**

The  $\alpha$ S1 fraction of casein can be obtained from milk proteins by various methods [D. G. Schmidh and T. A. J. Paynes (1963), Biochim., Biophys. Acta, 78:492; M. P. Thompson and C. A. Kiddy (1964), J. Dairy Sci.,

47:626; J. C. Mercier, *et al.* (1968), Bull. Soc. Chim. Biol. 50:521], and the complete amino acid sequence of the  $\alpha$ S1 fraction of casein was determined by J. C. Mercier *et al.* (1971) (Eur. J. Biochem. 23:41). The genomic and coding sequences of bovine  $\alpha$ S1 fraction of casein have also been cloned and sequenced employing recombinant DNA techniques [D. Koczan, *et al.* (1991), Nucl. Acids Res. 19(20): 5591; McKnight, R. A., *et al.* (1989), J. Dairy Sci. 72:2464-73]. Proteolytic cleavage and identification of N-terminal fragments from the  $\alpha$ S1 fraction of casein has been documented [J. C. Mercier, *et al.* (1970), Eur. J. Biochem. 16:439; P. L. H. McSweeney *et al.* (1993), J. Dairy Res., 60:401], as has the intestinal absorption and appearance of this fragment in mammalian plasma following ingestion of whole milk proteins [Fiat, A.M., *et al.* (1998) Biochimie, 80(2):2155-65]. Meisel, H. and Bockelmann, W. [(1999), Antonie Van Leeuwenhoek, 76:207-15] detected amino acid sequences of immunopeptides, casokinins and casomorphins in peptides liberated by lactic acid bacteria digests of  $\alpha$  and  $\beta$  casein fractions. Of particular interest is the anti-aggregating and thrombolytic activity demonstrated for C-terminal portions of the  $\alpha$ - and  $\kappa$ -casein fractions [Chabance, B. *et al.* (1997), Biochem. Mol. Biol. Int. 42(1) 77-84; Fiat AM. *et al.* (1993), J. Dairy Sci. 76(1): 301-310].

The coding sequences for bovine  $\alpha$ S2-,  $\beta$ - and  $\kappa$ -casein have also been cloned (Groenen *et al.*, Gene 1993; 123:187-93, Stewart, *et al.*, Mol. Biol Evol. 1987;4:231-41, and Stewart, *et al.*, Nucl Acids Res 1984;12:3895-907). The  $\alpha$ S2-casein coding sequence has numerous Alu-like retroposon sequences, and, although the gene is organized similarly to the  $\alpha$ S1-casein gene, sequence analysis indicates that it is more closely related to the  $\beta$ -casein-encoding gene.  $\beta$ -casein is characterized by numerous clusters of serine residues, which, when phosphorylated, can interact with and sequester calcium phosphate (Stewart *et al.*, Mol Biol Evol. 1987;4:231-43).  $\kappa$ -casein is a smaller polypeptide, the amino acid and nucleotide sequence of which (Alexander *et al.*, Eu. J. Biochem 1988;178:395-401) indicates that it is evolutionarily unrelated to the calcium-



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sensitive casein gene family. In the gut,  $\kappa$ -casein is split into an insoluble peptide (para-kappa casein) and a soluble hydrophilic glycopeptide (caseinomacropptide), which has been shown to be active in efficiency of digestion, prevention of neonate hypersensitivity to ingested proteins, and inhibition of gastric bacterial pathogens (Malkoski, et al, Antimicrob Agents Chemother, 2001;45:2309-15).

Previous studies have documented potential bioactive peptides encrypted in the N-terminal  $\alpha$ S1 casein, the  $\alpha$ S2-casein,  $\beta$ - casein and in the  $\kappa$ -casein amino acid sequences, but no mention was made of use of these protein fragments, specific sequences or defined synthetic peptides, alone or in combination, to enhance hematopoiesis, prevent viral infection or modulate the development of autoimmune diseases.

The present invention successfully addresses the shortcomings of the presently known art by providing peptides, and combinations thereof for the treatment of human disease, which peptides are derived from the N terminus portion of  $\alpha$ S1 casein,  $\alpha$ S2-casein,  $\beta$ -casein and  $\kappa$ -casein, alone or in combination, and posses no detectable toxicity and a high therapeutic efficacy in a broad variety of pathological indications.

#### 20 SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a method of preventing or treating an autoimmune or infectious disease or condition, the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

25 According to further features in preferred embodiments of the invention described below the autoimmune or infectious disease or condition is selected from the group consisting of a viral disease, a viral infection, AIDS, and infection by HIV.

According to another aspect of the present invention there is provided a method of preventing or treating a blood disease or condition, the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

5 According to further features in preferred embodiments of the invention described below the blood disease or condition is selected from the group consisting of thrombocytopenia, pancytopenia, granulocytopenia, an erythropoietin treatable condition, and a thrombopoietin treatable condition.

10 According to a yet another aspect of the present invention there is provided a method of modulating blood cell formation, the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

15 According to further features in preferred embodiments of the invention described below the modulating blood cell formation is selected from the group consisting of inducing hematopoiesis, inducing hematopoietic stem cells proliferation, inducing hematopoietic stem cells proliferation and differentiation, inducing megakaryocytopoiesis, inducing erythropoiesis, inducing leukocytopoiesis, inducing thrombocytopoiesis, inducing plasma cell proliferation, inducing dendritic cell proliferation and inducing macrophage  
20 proliferation.

According to still another aspect of the present invention there is provided a method of enhancing peripheral stem cell mobilization, the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

25 According to another aspect of the present invention there is provided a method of preventing or treating a metabolic disease or condition, the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

30 According to further features in preferred embodiments of the invention described below the metabolic disease or condition is selected from the group

consisting of NIDDM, IDDM, glucosuria, hyperglycemia, hyperlipidemia, and hypercholesterolemia.

According to another aspect of the present invention there is provided a method of preventing or treating conditions associated with myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT), the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

According to yet another aspect of the present invention there is provided a method of augmenting the effect of a blood cell stimulating factor, the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

According to further features in preferred embodiments of the invention described below the blood cell stimulating factor is selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

According to still another aspect of the present invention there is provided a method of enhancing colonization of donated blood stem cells in a myeloablated recipient, the method effected by treating a donor of the donated blood stem cells with a therapeutically effective amount of peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof prior to donation and implanting the donated blood stem cells in the recipient.

According to further features in preferred embodiments of the invention described below the method further comprising treating the donated blood cells with a blood cell stimulating factor, the blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF) prior to implanting the blood stem cells in the recipient.

According to yet another aspect of the present invention there is provided a method of enhancing colonization of donated blood stem cells in a myeloablated recipient, the method effected by treating the donated blood stem cells with a therapeutically effective amount of peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof prior to implanting the donated blood stem cells in the recipient.

According to further features in preferred embodiments of the invention described below the method further comprising treating the donor with a blood cell stimulating factor, the blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF) prior to donation and implanting the blood stem cells in the recipient.

According to still another aspect of the present invention there is provided a method of enhancing colonization of blood stem cells in a myeloablated recipient, the method effected by treating the blood stem cells with a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof prior to implanting the blood stem cells in the recipient.

According to further features in preferred embodiments of the invention described below the method further comprising treating the blood stem cells with a blood cell stimulating factor, the blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF) prior to implanting the blood stem cells in the recipient.

According to another aspect of the present invention there is provided a method for preventing or treating a condition associated with a SARS infective agent, the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

According to further features in preferred embodiments of the invention described below the SARS infective agent is a coronavirus.

According to further features in preferred embodiments of the invention described below the coronavirus is SARS-CoV.

According to another aspect of the present invention there is provided a method for preventing or treating a bacterial disease or condition, the method effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

According to further features in preferred embodiments of the invention described below the peptide is a fragment derived from by fragmentation of  $\alpha$  S1 casein.

According to yet further features in preferred embodiments of the invention described below the peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof is a synthetic peptide.

According to still further features in preferred embodiments of the invention described below the peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof has a sequence as set forth in one of SEQ ID NOs: 1-33.

According to further features in preferred embodiments of the invention described below the combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof is a mixture of peptides.

According to yet further features in preferred embodiments of the invention described below the combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

According to still further features in preferred embodiments of the invention described below the chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

According to further features in preferred embodiments of the invention described below the method further comprising administering to the subject in need thereof an effective amount of a blood cell stimulating factor, the blood

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cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

According to further features in preferred embodiments of the invention described below the method further comprising administering to the subject in  
5 need thereof an effective amount of erythropoietin, thrombopoietin or granulocyte colony stimulating factor (G-CSF).

According to one aspect of the present invention there is provided a pharmaceutical composition for preventing or treating an autoimmune or infectious disease or condition, the pharmaceutical composition comprising, as  
10 an active ingredient, a peptide derived from the N terminus portion of  $\alpha$ S1 casein and a pharmaceutically acceptable carrier.

According to further features in preferred embodiments of the invention described below the autoimmune or infectious disease or condition is selected from the group consisting of a viral disease, a viral infection, AIDS, and  
15 infection by HIV.

According to another aspect of the present invention there is provided a pharmaceutical composition for preventing or treating a blood disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a  
20 pharmaceutically acceptable carrier.

According to further features in preferred embodiments of the invention described below the blood disease or condition is selected from the group consisting of thrombocytopenia, pancytopenia, granulocytopenia, an erythropoietin treatable condition, and a thrombopoietin treatable condition and  
25 a granulocyte colony stimulating factor treatable condition.

According to yet another aspect of the present invention there is provided a pharmaceutical composition for modulating blood cell formation, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically  
30 acceptable carrier.

According to further features in preferred embodiments of the invention described below, modulating blood cell formation is selected from the group consisting of inducing hematopoiesis, inducing hematopoietic stem cells proliferation, inducing hematopoietic stem cells proliferation and  
5 differentiation, inducing megakaryocytopoiesis, inducing erythropoiesis, inducing leukocytopoiesis, inducing thrombocytopoiesis, inducing granulocytopoiesis, inducing plasma cell proliferation, inducing dendritic cell proliferation and inducing macrophage proliferation.

According to still another aspect of the present invention there is  
10 provided a pharmaceutical composition for enhancing peripheral stem cell mobilization, the pharmaceutical composition comprising, as an active ingredient, a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

According to another aspect of the present invention there is provided a  
15 pharmaceutical composition for preventing or treating a metabolic disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

According to further features in preferred embodiments of the invention  
20 described below the metabolic disease or condition is selected from the group consisting of NIDDM, IDDM, glucosuria, hyperglycemia, hyperlipidemia, and hypercholesterolemia.

According to yet another aspect of the present invention there is  
provided a pharmaceutical composition for preventing or treating conditions  
25 associated with myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT), the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

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According to still another aspect of the present invention there is provided a pharmaceutical composition for augmenting the effect of a blood cell stimulating factor, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and  
5 a pharmaceutically acceptable carrier.

According to further features of preferred embodiments in the invention described below, the blood cell stimulating factor is selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

10 According to another aspect of the present invention there is provided a pharmaceutical composition for enhancing colonization of donated blood stem cells in a myeloablated recipient, the pharmaceutical composition comprising, as active ingredients, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

15 According to yet another aspect of the present invention there is provided a pharmaceutical composition for enhancing colonization of blood stem cells in a myeloablated recipient, the pharmaceutical composition comprising as active ingredients, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

20 According to still another aspect of the present invention there is provided a pharmaceutical composition for treating or preventing an indication selected from the group consisting of autoimmune disease or condition, viral disease, viral infection, hematological disease, hematological deficiencies, thrombocytopenia, pancytopenia, granulocytopenia, hyperlipidemia,  
25 hypercholesterolemia, glucosuria, hyperglycemia, diabetes, AIDS, HIV-1, helper T-cell disorders, dendrite cell deficiencies, macrophage deficiencies, hematopoietic stem cell disorders including platelet, lymphocyte, plasma cell and neutrophil disorders, pre-leukemic conditions, leukemic conditions, immune system disorders resulting from chemotherapy or radiation therapy,  
30 human immune system disorders resulting from treatment of diseases of



immune deficiency and bacterial infections, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

According to another aspect of the present invention there is provided a pharmaceutical composition for treating or preventing an indication selected from the group consisting of hematological disease, hematological deficiencies, thrombocytopenia, pancytopenia, granulocytopenia, dendrite cell deficiencies, macrophage deficiencies, hematopoietic stem cell disorders including platelet, lymphocyte, plasma cell and neutrophil disorders, pre-leukemic conditions, leukemic conditions, myelodysplastic syndrome, non-myeloid malignancies, aplastic anemia and bone marrow insufficiency, the pharmaceutical composition comprising, as active ingredients, a blood cell stimulating factor and a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

According to one aspect of the present invention there is provided a purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-33.

According to another aspect of the present invention there is provided a pharmaceutical composition comprising a purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-33 and a pharmaceutically acceptable carrier.

According to another aspect of the present invention there is provided a purified chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

According to yet another aspect of the present invention there is provided a pharmaceutical composition comprising a purified chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage and a pharmaceutically acceptable carrier.

According to further features in preferred embodiments of the invention described below, the chimeric peptide comprising a first  $\alpha$ S1 casein peptide

having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

According to yet another aspect of the present invention there is provided a pharmaceutical composition comprising a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF), in combination with a purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-33 and a pharmaceutically acceptable carrier.

According to still another aspect of the present invention there is provided a pharmaceutical composition for preventing or treating a condition associated with a SARS infective agent, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

According to further features in preferred embodiments of the invention described below the SARS infective agent is a coronavirus.

According to still further features in preferred embodiments of the invention described below the coronavirus is SARS-CoV.

According to another aspect of the present invention there is provided a pharmaceutical composition for preventing or treating a bacterial infection the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

According to further features in preferred embodiments of the invention described below the peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

According to yet further features in preferred embodiments of the invention described below the peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof is a synthetic peptide.

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According to still further features in preferred embodiments of the invention described below the peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or has a sequence as set forth in one of SEQ ID NOs: 1-33.

According to further features in preferred embodiments of the invention described below the combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

According to further features in preferred embodiments of the invention described below the combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

According to yet further features in preferred embodiments of the invention described below the chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

According to still further features in preferred embodiments of the invention described below the pharmaceutical composition further comprising, as an active ingredient, a blood cell stimulating factor, the blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

According to further features in preferred embodiments of the invention described below the pharmaceutical composition further comprising, as an active ingredient, thrombopoietin, erythropoietin or granulocyte colony stimulating factor (G-CSF).

According to still another aspect of the present invention there is provided a method of low-temperature processing of casein proteolytic hydrolysate, the method effected by obtaining a casein proteolytic hydrolysate comprising proteolytic enzymes, cooling the casein proteolytic hydrolysate so as to inactivate the proteolytic enzymes, adjusting the pH of the casein protein hydrolysate to an acid pH, filtering the acidic casein protein hydrolysate,

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collecting the filtrate, and further acidifying the filtrate so as to precipitate proteins derived from natural casein, separating and collecting the precipitate, adjusting the pH of the precipitate to an alkaline pH so as to irreversibly inactivate the proteolytic enzymes; and adjusting the pH of the precipitate to pH  
5 7-9, thereby processing the casein protein hydrolysate at low temperature.

According to another aspect of the present invention there is provided a casein protein hydrolysate processed at low temperature according to the abovementioned method.

According to further features in preferred embodiments of the invention  
10 described below, step b comprises cooling to about 10°C.

According to still further features in preferred embodiments of the invention described below adjusting the pH of step c comprises addition of acid to 2% (w/v) acid, and the further acidifying the filtrate of step d comprises additional addition of acid to about 10% (w/v) acid.

15 According to yet further features in preferred embodiments of the invention described below the alkaline pH of step f is at least pH 9.

The present invention successfully addresses the shortcomings of the presently known configurations by providing peptides for the treatment of human disease, which peptides are derived from the N terminus portion of  $\alpha$ S1  
20 casein,  $\alpha$ S2-casein,  $\beta$ -casein and  $\kappa$ -casein, alone or in combination and possess no detectable toxicity and high therapeutic efficacy.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with  
25 reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood  
30 description of the principles and conceptual aspects of the invention. In this

regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

5 In the drawings:

FIG. 1 depicts the stimulation of Natural Killer (NK) cell activity in cultured murine bone marrow cells by peptides derived from natural casein. Lysis of  $^{35}\text{S}$  labeled YAC target cells by cultured murine bone marrow cells incubated in the presence or absence of 100  $\mu\text{g}$  per ml peptides derived from natural casein is expressed as the fraction of total radioactivity released from the YAC cells into the culture supernatant (% Release  $^{35}\text{S}$ ). Figure 1 represents NK activity at an effector:target cell ratio of 25:1 and 50:1.

FIGs. 2a and 2b depict the stimulation of Natural Killer (NK) cell activity in cultured human Peripheral Blood Stem Cells (PBSC) by peptides derived from natural casein. Lysis of  $^{35}\text{S}$  labeled K562 target cells by cultured human PBSC from Granulocyte Colony Stimulating Factor (G-CSF) treated donors incubated without (0  $\mu\text{g}$ ) or with increasing concentrations (5– 500  $\mu\text{g}$  per ml) of peptides derived from natural casein is expressed as the fraction of total radioactivity released from the K562 cells into the culture supernatant (% Release  $^{35}\text{S}$ ). Figure 2a represents NK activity of two blood samples from the same patient, incubated at different effector:target cell ratios (100:1 and 50:1). Figure 2b represents NK activity of blood samples from normal and affected donors incubated at a 100:1 effector:target cell ratio. Squares represent an effector:target cell ratio of 100:1, diamonds represent an effector:target cell ratio of 50:1.

FIGs. 3a:3c depict the stimulation of proliferation of Natural Killer (NK) and T-lymphocyte (T) cells from cultured human Peripheral Blood Stem Cells (PBSC) by peptides derived from natural casein. NK and T cell proliferation in cultured PBSC from Granulocyte Colony Stimulating Factor treated donors

incubated with or without peptides derived from natural casein is expressed as the percentage (%) of cells binding the anti-CD<sub>3</sub>/FITC fluorescent anti-T cell antibody UCHT<sub>1</sub>, or the anti CD<sub>56</sub>/RPE fluorescent anti-NK cell antibody MOC-1 (DAKO A/S Denmark). Controls are FITC and RPE-conjugated anti-mouse IgG antibody. Figure 3a represents the percentage of cultured human PBSC binding fluorescent antibody CD<sub>56</sub> (5 independent samples) after 10 days incubation with (peptides) or without (control) 100 µg per ml peptides derived from natural casein. Figure 3b represents the percentage of cultured human PBSCs binding fluorescent anti-CD<sub>3</sub> (T cell) antibody, following 14 days of incubation with (peptides) or without (control) 100 µg per ml peptides derived from natural casein. Figure 3c represents the percentage of cultured human PBSCs binding fluorescent anti-CD<sub>3</sub> (T cell) antibody and cells binding both CD<sub>3</sub> and CD<sub>56</sub> (T and NK-like cells) antibodies after 28 days incubation with (peptides) or without (control) 100 µg per ml peptides derived from natural casein.

FIG. 4 depicts the stimulation of Natural Killer (NK) cell activity in cultured human Peripheral Blood Stem Cells (PBSC) by synthetic peptides derived from αS1-casein. Lysis of <sup>35</sup>S labeled K562 target cells by cultured human PBSC (from a breast cancer patient) incubated without (0 µg) or with increasing concentrations (10 - 500 µg per ml) of synthetic peptides derived from casein is expressed as the fraction of total radioactivity released from the K562 cells into the culture supernatant (% Release). Peptides represent N-terminal sequences of 1-10 (1a, diamonds), 1-11 (2a, squares) and 1-12 (3a, triangles) first amino acids of the N terminus portion of αS1 casein (see Table 3 below for sequences of synthetic peptides).

FIGs. 5a-5c depict the stimulation of proliferation of cultured human cells of diverse origin by peptides derived from natural casein. Proliferation of the cultured human cells after 14-21 days incubation with increasing concentrations of the peptides derived from natural casein is expressed as the

amount of [ $^3\text{H}$ ]-thymidine incorporated into each sample. Figure 5a represents the incorporation of label into two samples (PBSC 1, squares, 15 days incubation; and PBSC 2, diamonds, 20 days incubation) of human Peripheral Blood Stem Cells incubated with or without (ctrl) 50 - 600  $\mu\text{g}$  per ml peptides derived from natural casein. Figure 5b represents the incorporation of [ $^3\text{H}$ ]-thymidine into cultured human bone marrow cells after 21 days incubation with or without (ctrl) 50 - 600  $\mu\text{g}$  per ml peptides derived from natural casein. Bone marrow was donated by cancer patients in remission (BM Auto, closed squares, BM 1, triangles, and BM 2, -open squares-) or healthy volunteers (BM normal, diamonds). Figure 5c represents incorporation of [ $^3\text{H}$ ]-thymidine into cultured human Cord Blood cells after 14 days incubation with or without (ctrl) 50-1000  $\mu\text{g}$  per ml peptides derived from natural casein. Cord blood cells were donated by two separate donors (C.B. 1, triangles, C.B. 2, squares).

FIG. 6 shows a Table depicting the proliferation of blood cell progenitors from human bone marrow and cord blood in response to incubation with peptides derived from natural casein. The relative cell number  $\times 10^4$  per ml, reflecting the proliferation of cultured cells, was determined by counting cells as described in the Examples section that follows. Bone marrow from healthy volunteers (Bone Marrow) and Cord Blood from normal births (Cord Blood) was incubated for 13 (Cord Blood) or 14 (Bone Marrow) days in the presence of growth factors and AB serum, with or without increasing concentrations of peptides derived from natural casein (25-500  $\mu\text{g}/\text{ml}$ ).

FIG. 7 shows a table depicting the effect of in-vitro incubation with synthetic peptides derived from  $\alpha\text{S1}$ -casein on the relative distribution of Megakaryocyte, Erythroid, Plasma and Dendritic cells (differential count) in CFU-GEMM colonies from murine bone marrow progenitor cells. Cells were scored in the macroscopic colonies grown from murine bone marrow cells prepared similarly to the CFU-GEMM colonies. Cells were incubated with hematopoietic factors, and 25  $\mu\text{g}$  or more of Synthetic peptides derived from

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casein, for 14 days. The differential count is expressed as the percentage of total cells represented by individual cell types.

FIG. 8 depicts the stimulation of peripheral white blood cell reconstitution in myeloablated, bone marrow transplanted mice in response to treatment with peptides derived from natural casein. Cell counts represent the number of white blood cells ( $\times 10^4$  per ml, as counted in a haemocytometer). The mice ( $n = 6$  per group) received sub-lethal irradiation and syngeneic bone marrow transplantation ( $10^6$  cells per mouse) on the following day, and intravenous administration of 1 mg per recipient peptides derived from natural casein (peptides: squares) or 1 mg per recipient human serum albumin (CONTROL: diamonds) one day later.

FIG. 9 depicts the stimulation of platelet reconstitution in myeloablated, bone marrow transplanted mice in response to treatment with peptides derived from natural casein. Platelet (PLT) counts represent the number of thrombocytes ( $\times 10^6$  per ml, as counted in a haemocytometer). The mice ( $n = 7$  or 10 per group) received lethal irradiation and syngeneic bone marrow transplantation ( $10^6$  cells per mouse) on day 1, and intravenous administration of 1 mg per recipient peptides derived from natural casein (Peptides, diamonds) or 1 mg per recipient human serum albumin (control, squares).

FIGs. 10a-10f depict the penetration and nuclear uptake of FITC-conjugated peptides derived from natural casein in cultured human T-lymphocyte cells, as recorded by fluorescent microscopy. Sup-T1 cells were incubated with 100  $\mu$ g per ml FITC-conjugated peptides derived from natural casein as described in the Examples section that follows. At the indicated times, the cells were washed of free label, fixed in formalin and prepared for viewing and recording by Laser Scanning Confocal Microscopy. Figures 10a through 10f are selected images of cells from consecutive incubation times, demonstrating FITC-conjugated peptides derived from natural casein penetrating the Sup-T1 cell membrane (Figures 10a, 10b) and concentrating in the nucleus (Figures 10c- 10f).



FIG. 11 shows a Table depicting the stimulation of Sup-T<sub>1</sub> Lymphocyte cell proliferation in response to incubation with peptides derived from natural casein. Sup-T<sub>1</sub> cells (5000 per well) were incubated with increasing concentrations (50 - 1000 µg per ml) of peptides derived from natural casein, counted in their wells at the indicated times post culture and pulsed with [<sup>3</sup>H]-thymidine for 18 hours. Proliferation index is the ratio of the average of the incorporation of [<sup>3</sup>H]-thymidine into cells cultured with peptides derived from natural casein (triplicate samples) divided by the incorporation into cells cultured without peptides derived from natural casein (control).

FIG. 12 shows a Table depicting inhibition of HIV-1 infection of CEM lymphocytes by peptides derived from natural casein. CEM cells were either contacted with HIV-1 virus preincubated 3 hours with peptides derived from natural casein (3 hours), or preincubated themselves with increasing concentrations (50 - 1000 µg per ml) of peptides derived from natural casein for the indicated number of hours (24 and 48 hours) before contact with HIV-1 virus, as described in the Examples section that follows. On day 15 post infection, cells were counted for cell numbers and assayed for severity of HIV-1 infection by the P<sup>24</sup> antigen assay, as described in the Examples section that follows. Control cultures were IF: CEM cells contacted with HIV-1 virus without pretreatment with peptides derived from natural casein, and UIF: CEM cells cultured under identical conditions without peptides derived from natural casein and without contact with HIV-1 virus.

FIG. 13 shows a Table depicting inhibition of HIV-1 infection of CEM lymphocytes by synthetic peptides derived from αS1-casein. CEM cells were contacted with HIV-1 virus which had been preincubated with various concentrations (10- 500 µg per ml) of synthetic peptides derived from αS1-casein (1P, 3P and 4P) for 3 hours (in the presence of the peptides), as described in the Examples section that follows. On day 7 post infection, cells were counted for cell numbers and assayed for severity of HIV-1 infection by

the  $P^{24}$  antigen assay, as described in the Examples section that follows. Control cultures (IF) were CEM cells contacted with HIV-1 virus without pretreatment with synthetic peptides derived from  $\alpha S1$ -casein, and UIF: CEM cells cultured under identical conditions without synthetic peptides derived from casein and without contact with HIV-1 virus.

FIG. 14 depicts the prevention by peptides derived from natural casein of Type I (IDDM) Diabetes in female Non Obese Diabetic (NOD) mice. Glucosuria was monitored at intervals during 365 days post treatment in female NOD mice receiving a once (triangles) or twice (squares) weekly injection of 100  $\mu$ g peptides derived from natural casein for 5 weeks (5 or 10 injections total) and untreated controls. All the controls developed glucosuria and subsequently died.

FIG. 15 depicts the reduction by synthetic peptides derived from  $\alpha S1$ -casein of diet-induced hypercholesterol/hyperlipidemia in female C57Bl/6 mice. Total cholesterol (TC), High Density (HDL) and Low Density Lipoproteins (LDL) were assayed in pooled blood of two (2) mice per sample from hypercholesterol/hyperlipidemic mice receiving (IP) casein-derived peptides B, C, 2a or 3P, or no treatment (control). "Normal" samples represent control mice not fed the atherogenic diet.

FIG. 16 shows a Table depicting the stimulation of hematopoiesis in cancer patients in response to injections of peptides derived from natural casein. Peripheral blood from five female cancer patients either receiving or having received chemotherapy, as described above, was counted for total White Blood Cells (WBC,  $\times 10^3$ ), Platelets (PLT,  $\times 10^6$ ), Erythrocytes (RBC,  $\times 10^3$ ) and Hemoglobin (gm per dl) before (n) and after (n + ...) intramuscular injections with peptides derived from natural casein. Patient 1 relates to G.T.; patient 2 relates to E.C.; patient 3 relates to E.S.; patient 4 relates to J.R. and patient 5 relates to D.M.

FIG. 17 depicts the stimulation by peptides derived from natural casein of thrombocytopoiesis in a platelet-resistant patient with Acute Myeloid Leukemia (M-1). Thrombocyte reconstitution was expressed as the change in platelet content of peripheral blood (PLT,  $\times 10^6$  per ml), counted as described above at the indicated

intervals following intramuscular injection (as described in the Examples section that follows) of 100 mg peptides derived from natural casein.

FIG. 18 depicts the stimulation by peptides derived from natural casein of thrombocytopoiesis in a platelet-resistant patient with Acute Myeloid Leukemia (M-2). Thrombocyte reconstitution was expressed as the change in platelet content of peripheral blood (PLT,  $\times 10^6$  per ml), counted as described above at the indicated intervals following intramuscular injection (as described in the Examples section that follows) of 100 mg peptides derived from natural casein.

FIG. 19 shows a table depicting the synergistic effect of incubation with synthetic peptides derived from  $\alpha$ S1-,  $\alpha$ S2-,  $\beta$ - or  $\kappa$ -casein on hematopoietic factor stimulation of granulocyte and monocyte colony formation in CFU-GM colonies from murine bone marrow progenitor cells. Cells were scored in the macroscopic colonies grown from murine bone marrow cells prepared similarly to the CFU-GEMM colonies previously described. Cells were incubated with hematopoietic factors cytokine (IL-3) and colony stimulating factor (G-CSF), and 25  $\mu$ g or more of synthetic peptides derived from casein (J), representing amino acids 1-22 of  $\alpha$ -S1 casein (SEQ ID No. 21), or 30-4, representing amino acids 1-6 of  $\alpha$ -S1 casein (SEQ ID No. 5), for 14 days, individually or in combination. The stimulation of colony formation (CFU) is expressed as the number of myeloid per colonies in  $10^5$  plated MNCs. Note the synergistic increase in myelocyte formation in cultures exposed to G-CSF, IL-3 and either of the synthetic peptides derived from casein.

FIG. 20 shows a table depicting the synergistic effect of incubation with synthetic peptides derived from  $\alpha$ S1-,  $\alpha$ S2-,  $\beta$ - or  $\kappa$ -casein on hematopoietic factor stimulation of granulocyte and monocyte colony formation in CFU-GM colonies from human bone marrow progenitor cells. Cells were scored in the macroscopic colonies grown from human bone marrow cells prepared similarly to the CFU-GEMM colonies previously described. Cells were incubated with hematopoietic factors cytokine (IL-3) and colony stimulating factor (G-CSF), and 25  $\mu$ g or more of synthetic peptides derived from casein: peptide J,

representing amino acids 1-22 of  $\alpha$ -S1 casein (SEQ ID NO. 21), or  $\beta$ -casein, representing amino acids 193-208 of  $\beta$ -casein (SEQ ID No. 28). Exposure of the human bone marrow progenitor cells to peptides derived from casein was for 14 days. Stimulation of colony formation (CFU) is expressed as the number of myeloid per colonies in  $10^5$  plated MNCs. Note the synergistic increase (> 50% with 100  $\mu$ g/ml of peptide J, and > 30% with 300  $\mu$ g/ml of synthetic  $\beta$ -casein) in myelocyte formation in cultures exposed to G-CSF, IL-3 and the synthetic peptides derived from  $\beta$ -casein and an N terminal portion of  $\alpha$ -S1 casein.

FIG. 21 shows a table depicting the effect of incubation with synthetic peptides derived from  $\alpha$  S1-,  $\alpha$ S2-,  $\beta$ - or  $\kappa$ -casein on Megakaryocytopoiesis in CFU-GEMM colonies from murine bone marrow progenitor cells. Cells were scored in the macroscopic colonies grown from murine bone marrow cells prepared similarly to the CFU-GEMM colonies previously described. Cells were incubated with 25  $\mu$ g or more of synthetic peptides derived from casein : synthetic  $\beta$ -casein (SEQ ID NO: 28), synthetic  $\kappa$ -casein (SEQ ID NO: 30), and synthetic peptides derived from casein representing amino acids 1-22 of  $\alpha$ -S1 casein (J) (SEQ ID NO:21), for 14 days. Stimulation of megakaryocyte formation is expressed as the percent of megakaryocytes (differential count). Note the dramatic effect of the synthetic peptides derived from  $\alpha$ S1-,  $\beta$ - or  $\kappa$ -casein on Early (E.MK) megakaryocyte formation.

FIG. 22 shows a table depicting the effect of in-vitro incubation with peptides derived from  $\alpha$ S1-,  $\alpha$ S2-,  $\beta$ - or  $\kappa$ -casein on the growth of GEMM colonies from murine bone marrow progenitor cells. Cells were scored in the macroscopic colonies grown from murine bone marrow cells prepared similarly to the CFU-GEMM colonies previously described. Cells were incubated with hematopoietic factors, and 25  $\mu$ g/ml of synthetic  $\beta$ -casein (193-208) (SEQ ID NO: 28) or synthetic  $\kappa$ -casein (106-127) (SEQ ID NO: 30), or a combination of both synthetic ( $\beta$  +  $\kappa$ ), for 8 days. The stimulation of colony formation is expressed as the number of CFU-GEMM colonies as compared to controls.

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Note the significant effect of both the synthetic  $\beta$ - and synthetic  $\kappa$ -casein peptides on GEMM colony formation, and the synergistic effect of both synthetic  $\beta$ - and synthetic  $\kappa$ -casein in combination.

FIG. 23 shows a table depicting the stimulation of platelet reconstitution in myeloablated, bone marrow transplanted mice in response to treatment with synthetic peptides [ $\beta$  casein (193-208) (SEQ ID NO: 28) and  $\kappa$ -casein (106-127) (SEQ ID NO: 30)] and synthetic  $\alpha$ -S1 casein [peptide J, (SEQ ID NO: 21) representing amino acids 1-22 of  $\alpha$ -S1 casein]. Cell counts represent the number of platelets ( $\times 10^3$  per  $\text{mm}^3$ , as counted in a Coulter Counter). The mice (n = 5 per group) received sub-lethal irradiation and syngeneic bone marrow transplantation ( $3 \times 10^6$  cells per mouse) on the following day, and intravenous administration of 1 mg per recipient of synthetic  $\beta$ -casein ; synthetic  $\kappa$ -casein, or synthetic peptide J (SEQ ID NO: 21) representing amino acids 1-22 of  $\alpha$ -S1 casein, or 1 mg per recipient human serum albumin (CONTROL) one day later. Platelets were measured 10 days later. Note the strong effect ( $> 25\%$  enhancement) of the synthetic  $\beta$ -casein,  $\kappa$ -casein and synthetic peptide J on platelet reconstitution at 10 days post ablation.

FIG. 24. depicts the stimulation of peripheral white blood cell reconstitution in myeloablated, bone marrow transplanted mice in response to treatment with peptides derived from  $\alpha$ S1-,  $\beta$ - or  $\kappa$ -casein. Cell counts represent the mean values of white blood cells (per ml, as counted in a haemocytometer). The mice (n = 5 per group) received sub-lethal irradiation and syngeneic bone marrow transplantation ( $3 \times 10^6$  cells per mouse) on the following day, and intravenous administration of 1 mg per recipient of  $\alpha$ -S1 or  $\kappa$  peptides derived from natural casein prepared from gel filtration ( $\alpha$ -S1 1-23 and  $\kappa$  106-169), synthetic peptides derived from  $\alpha$ -S1 casein (SEQ ID NO: 21) or  $\beta$ - casein (193-208, SEQ ID NO: 28), or 1 mg per recipient human serum albumin (CONTROL) one day later. Note the dramatic enhancement of white blood cell reconstitution by peptides derived from  $\alpha$ S1-,  $\beta$ - or  $\kappa$ -casein at days 5 and 7 post-reconstitution.

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FIG. 25 depicts the stimulation of peripheral white blood cell reconstitution in myeloablated, bone marrow transplanted mice in response to treatment with a combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein. Cell counts represent the mean values of white blood cells ( $\times 10^4$  per ml, as counted in a haemocytometer). The mice ( $n = 5$  per group) received sub-lethal irradiation and syngeneic bone marrow transplantation ( $10^6$  cells per mouse) on the following day, and intravenous administration of 1 mg per recipient of synthetic peptides derived from  $\alpha$ -S1 casein (J, SEQ ID NO: 21) or  $\beta$ -casein (193-208, SEQ ID NO: 28), a combination thereof [0.5 mg each of  $\alpha$ -S1-(J) and  $\beta$ -casein] or saline (Saline) one day later. Note the dramatic enhancement of white blood cell reconstitution by the combination of peptides derived from  $\alpha$ -S1- and  $\beta$ -casein at days 10 and 12 post-reconstitution.

FIG. 26 is a table depicting a representative series of chimeric peptides comprising amino acid sequences of the N-terminal sequence of  $\alpha$ -S1-casein (SEQ ID NO: 25) and  $\beta$ -casein (SEQ ID NO: 28).

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of biologically active peptides that are derived from or are similar to sequences identical with the N-terminus of the  $\alpha$ -S1-fraction of milk casein, compositions containing same and methods of utilizing same in, for example, stimulating and enhancing immune response, protecting against viral infection, normalizing serum cholesterol levels, and stimulating hematopoiesis. The casein-derived peptides are non-toxic and can be used to treat and prevent, for example, immune pathologies, hypercholesterolemia, hematological disorders and viral-related diseases.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The

invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

5 As used herein, the term "treating" includes substantially inhibiting, slowing or reversing the progression of a disease, and/or substantially ameliorating clinical symptoms of a disease.

As used herein, the term "preventing" includes substantially preventing the appearance of clinical symptoms of a disease.

10 As used herein the term "peptide" includes native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptido-mimetics (typically, synthetically synthesized peptides), such as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body.

15 Such modifications include, but are not limited to, cyclization, N terminus modification, C terminus modification, peptide bond modification, including, but not limited to,  $\text{CH}_2\text{-NH}$ ,  $\text{CH}_2\text{-S}$ ,  $\text{CH}_2\text{-S=O}$ ,  $\text{O=C-NH}$ ,  $\text{CH}_2\text{-O}$ ,  $\text{CH}_2\text{-CH}_2$ ,  $\text{S=C-NH}$ ,  $\text{CH=CH}$  or  $\text{CF=CH}$ , backbone modification and residue modification. Methods for preparing peptido-mimetic compounds are well known in the art

20 and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further detail in this respect are provided hereinunder.

Thus, a peptide according to the present invention can be a cyclic

25 peptide. Cyclization can be obtained, for example, through amide bond formation, e.g., by incorporating Glu, Asp, Lys, Orn, di-amino butyric (Dab) acid, di-aminopropionic (Dap) acid at various positions in the chain ( $\text{-CO-NH}$  or  $\text{-NH-CO}$  bonds). Backbone to backbone cyclization can also be obtained through incorporation of modified amino acids of the formulas  $\text{H-N}((\text{CH}_2)_n\text{-}$

COOH)-C(R)H-COOH or H-N((CH<sub>2</sub>)<sub>n</sub>-COOH)-C(R)H-NH<sub>2</sub>, wherein n = 1-4, and further wherein R is any natural or non-natural side chain of an amino acid.

Cyclization via formation of S-S bonds through incorporation of two Cys residues is also possible. Additional side-chain to side chain cyclization  
5 can be obtained via formation of an interaction bond of the formula  $-(\text{-CH}_2\text{-})_n\text{-S-CH}_2\text{-C-}$ , wherein n = 1 or 2, which is possible, for example, through incorporation of Cys or homoCys and reaction of its free SH group with, e.g., bromoacetylated Lys, Orn, Dab or Dap.

Peptide bonds (-CO-NH-) within the peptide may be substituted, for  
10 example, by N-methylated bonds (-N(CH<sub>3</sub>)-CO-), ester bonds (-C(R)H-C-O-O-C(R)-N-), ketomethylene bonds (-CO-CH<sub>2</sub>-),  $\alpha$ -aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH<sub>2</sub>-NH-), hydroxyethylene bonds (-CH(OH)-CH<sub>2</sub>-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), peptide derivatives (-N(R)-CH<sub>2</sub>-CO-  
15 ), wherein R is the "normal" side chain, naturally presented on the carbon atom.

These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) at the same time.

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as TIC, naphthylelanine (Nol), ring-methylated  
20 derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

Tables 1-2 below list all the naturally occurring amino acids (Table 1) and non-conventional or modified amino acids (Table 2).

**Table 1**

Amino Acid	Three-Letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q



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Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

Table 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
$\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
carboxylate		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbomyl-	Norb	L-N-methylglutamine	Nmgin
carboxylate		L-N-methylglutamic acid	Nmghu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmbis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser

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D-lysine	Dlys	L-N-methylthreonine	Nmtthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgabv
D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchcxa
D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpen
D- $\alpha$ -methylasparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
D- $\alpha$ -methylaspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D- $\alpha$ -methylhistidine	Dmbis	N-(3-aminopropyl)glycine	Nom
D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
D- $\alpha$ -methylleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
D- $\alpha$ -methyllysine	Dmlys	N-benzylglycine	Nphe
D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngin
D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Ncbut
D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D- $\alpha$ -methyltyrosine	Dmty	N-cyclodecylglycine	Nedec
D- $\alpha$ -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
D- $\alpha$ -methylalanine	Dmala	N-cyclooctylglycine	Ncoct
D- $\alpha$ -methylarginine	Dmarg	N-cyclopropylglycine	Nopro

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D- $\alpha$ -methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D- $\alpha$ -methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nblm
D- $\alpha$ -methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbbe
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtp
D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmnet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nile	D-N-methylsarine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nva
D-N-methyltyrosine	Dnmtyr	N-methyl-naphthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
$\gamma$ -aminobutyric acid	Gabu	N-(p-hydroxyphenyl)glycine	Nhtyr
L-t-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	Penicillamine	Pen
L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methylasparagine	Masn
L- $\alpha$ -methylaspartate	Masp	L- $\alpha$ -methyl-t-butylglycine	Mtbug
L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
L- $\alpha$ -methylglutamine	Mgln	L- $\alpha$ -methylglutamate	Mglu
L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomo phenylalanine	Mhphe
L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtp
D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmnet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe

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N-methylaminoisobutyrate	Nnaib	D-N-methylproline	Dnmpo
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmsr
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyl- $\alpha$ -naphthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
$\gamma$ -aminobutyric acid	Gabu	N-(p-hydroxyphenyl)glycine	Nhtyr
L-t-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	Penicillamine	Pen
L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methylassparagine	Masn
L- $\alpha$ -methylasspartate	Masp	L- $\alpha$ -methyl-t-butylglycine	Mtbug
L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
L- $\alpha$ -methylglutamine	Mgin	L- $\alpha$ -methylglutamate	Mglu
L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomophenylalanine	Mhphe
L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L- $\alpha$ -methyllucine	Mleu	L- $\alpha$ -methyllysine	Mlys
L- $\alpha$ -methylmethionine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
L- $\alpha$ -methylvaline	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr
L- $\alpha$ -methyllucine	Mval	L-N-methylhomophenylalanine	Nmhphe
	Nabhm		
N-(N-(2,2-diphenylethyl)		N-(N-(3,3-diphenylpropyl)	
carbamylmethyl-glycine	Nabhm	carbamylmethyl(1)glycine	Nabhe
1-carboxy-1-(2,2-diphenyl ethylamino)cyclopropane	Nmbc		

A peptide according to the present invention can be used in a self standing form or be a part of moieties such as proteins and display moieties such as display bacteria and phages. The peptides of the invention can also be

chemically modified to give active dimers or multimers, in one polypeptide chain or covalently crosslinked chains.

Additionally, a peptide according to the present invention includes at least two, optionally at least three, optionally at least four, optionally at least five, optionally at least six, optionally at least seven, optionally at least eight, optionally at least nine, optionally at least ten, optionally at least eleven, optionally at least twelve, optionally at least thirteen, optionally at least fourteen, optionally at least fifteen, optionally at least sixteen, optionally at least seventeen, optionally at least eighteen, optionally at least nineteen, optionally at least twenty, optionally at least twenty-one, optionally at least twenty-two, optionally at least twenty-three, optionally at least twenty-four, optionally at least twenty-five, optionally at least twenty-six, optionally between twenty-seven and sixty, or more amino acid residues (also referred to herein interchangeably as amino acids).

Accordingly, as used herein the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

As used herein the phrase "derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein" refers to peptides as this term is defined herein, e.g., cleavage products of  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein (referred to herein as peptides derived from natural casein), synthetic peptides chemically synthesized to correspond to the amino acid sequence of  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein (referred to herein as synthetic peptides derived from casein), peptides similar (homologous) to  $\alpha$ S1-casein,  $\alpha$ S2-casein  $\beta$ -casein,  $\kappa$ -casein, for example, peptides characterized by one or more amino acid substitutions, such as, but not limited to, permissible substitutions, provided that at least 70 %, preferably at least 80 %, more preferably at least 90 % similarity is maintained,

and functional homologues thereof. The terms "homologues" and "functional homologues" as used herein mean peptides with any insertions, deletions and substitutions which do not affect the biological activity of the peptide.

As used herein, the phrase "peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein and combinations thereof" also refers to the abovementioned peptides in combination with one another. As used herein, the phrase "combination thereof" is defined as any of the abovementioned peptides, derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, combined in a mixture and/or chimeric peptide with one or more additional, non-identical peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein. As used herein, the term "mixture" is defined as a non-covalent combination of peptides existing in variable proportions to one another, whereas the term "chimeric peptide" is defined as at least two identical or non-identical peptides covalently attached one to the other. Such attachment can be any suitable chemical linkage, direct or indirect, as via a peptide bond, or via covalent bonding to an intervening linker element, such as a linker peptide or other chemical moiety, such as an organic polymer. Such chimeric peptides may be linked via bonding at the carboxy (C) or amino (N) termini of the peptides, or via bonding to internal chemical groups such as straight, branched or cyclic side chains, internal carbon or nitrogen atoms, and the like. According to a preferred embodiment of the present invention, the chimeric peptide comprises a peptide derived from an N terminus portion of  $\alpha$ -S1 casein as set forth in any of SEQ ID NOs:1-25 linked via the carboxy (C) terminal with the amino (N) terminal of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein as set forth in any of SEQ ID NOs: 1-33 and 434-4000. SEQ ID NOs: 434-4000 represent all possible peptides of at least 2 amino acids derived from the major and minor peptides derived from natural casein, as described hereinbelow (SEQ ID NOs: 25, and 27-33). It will be appreciated that, in further embodiments the chimeric peptides of the present invention can comprise all possible permutations of any of the peptides having an amino acid sequence as set forth in SEQ ID NOs: 1-33 and 34-4000, covalently linked to any other of the peptides having an amino acid sequence as

set forth in any of SEQ ID NOs: 1-33 and 34-4000. Such chimeric peptides can be easily identified and prepared by one of ordinary skill in the art, using well known methods of peptide synthesis and/or covalent linkage of peptides, from any of the large but finite number of combinations of peptides having an amino acid sequence as set forth in SEQ ID NOs: 1-33 and 434-4000. Non-limiting  
5 examples of such chimeric peptides comprising permutations of peptides derived from  $\alpha$ -S1 casein, as set forth in SEQ ID NOs: 1-25, covalently linked to peptides derived from  $\beta$ -casein, as set forth in SEQ ID NOs: 27 and 28, designated SEQ ID NOs: 34-433, are presented in Figure 26 hereinbelow.

10 The chimeric peptides of the present invention may be produced by recombinant means or may be chemically synthesised by, for example, the stepwise addition of one or more amino acid residues in defined order using solid phase peptide synthetic techniques. Where the peptides may need to be synthesised in combination with other proteins and then subsequently isolated  
15 by chemical cleavage or alternatively the peptides or polyvalent peptides may be synthesised in multiple repeat units. The peptides may comprise naturally occurring amino acid residues or may also contain non-naturally occurring amino acid residues such as certain D-isomers or chemically modified naturally occurring residues. These latter residues may be required, for example, to  
20 facilitate or provide conformational constraints and/or limitations to the peptides. The selection of a method of producing the subject peptides will depend on factors such as the required type, quantity and purity of the peptides as well as ease of production and convenience.

The chimeric peptides of the present invention may first require their  
25 chemical modification for use in vivo. Chemical modification of the subject peptides may be important to improve their biological activity. Such chemically modified chimeric peptides are referred to herein as "analogues". The term "analogues" extends to any functional chemical or recombinant equivalent of the chimeric peptides of the present invention, characterised, in a  
30 most preferred embodiment, by their possession of at least one of the

abovementioned biological activities. The term "analogue" is also used herein to extend to any amino acid derivative of the peptides as described above,

Analogues of the chimeric peptides contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptides or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with  $\text{NaBH}_4$ ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with  $\text{NaBH}_4$ .

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.



Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

5        Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

10        Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

15        As used herein the phrase "derived from an N terminus portion of  $\alpha$ S1 casein" refers to peptides as this term is defined herein, e.g., cleavage products of  $\alpha$ S1 casein (referred to herein as peptides derived from natural casein), synthetic peptides chemically synthesized to correspond to the amino acid sequence of an N terminus portion of  $\alpha$ S1 casein (referred to herein as synthetic peptides derived from casein), peptides similar (homologous) to an N terminus  
20        portion of  $\alpha$ S1 casein, for example, peptides characterized by one or more amino acid substitutions, such as, but not limited to, permissible substitutions, provided that at least 70 %, preferably at least 80 %, more preferably at least 90 % similarity is maintained, and functional homologues thereof. The terms  
25        "homologues" and "functional homologues" as used herein mean peptides with any insertions, deletions and substitutions that do not affect the biological activity of the peptide.

As used herein the phrase "derived from  $\alpha$ -,  $\beta$ - and  $\kappa$ -casein" refers to peptides as this term is defined herein, e.g., cleavage products of  $\alpha$ -,  $\beta$ - and  $\kappa$ -casein (referred to herein as peptides derived from natural casein), synthetic

peptides chemically synthesized to correspond to the amino acid sequence of  $\alpha$ -,  $\beta$ - and  $\kappa$ -casein (referred to herein as synthetic peptides derived from  $\alpha$ -,  $\beta$ - and  $\kappa$ -casein), peptides similar (homologous) to  $\alpha$ -,  $\beta$ - and  $\kappa$ -casein, for example, peptides characterized by one or more amino acid substitutions, such as, but not limited to, permissible substitutions, provided that at least 70 %, preferably at least 80 %, more preferably at least 90 % similarity is maintained, and functional homologues thereof. The terms "homologues" and "functional homologues" as used herein mean peptides with any insertions, deletions and substitutions that do not affect the biological activity of the peptide.

As used herein the terms " $\alpha$ -casein", " $\beta$ -casein" and " $\kappa$ -casein" refer to " $\alpha$ S1 casein", " $\alpha$ S2 casein", " $\beta$ -casein" and " $\kappa$ -casein" of a mammal, including, but not limited to, livestock mammals (e.g., cow, sheep, goat, mare, camel, deer and buffalo) human beings and marine mammals. The following provides a list of  $\alpha$ S1 caseins,  $\beta$ -caseins and  $\kappa$ -caseins having a known amino acid sequence, identified by their GenBank (NCBI) Accession Nos. and source:  $\alpha$ S1 caseins: CAA26982 (*Ovis aries* (sheep)), CAA51022 (*Capra hircus* (goat)), CAA42516 (*Bos taurus* (bovine)), CAA55185 (*Homo sapiens*), CAA38717 (*Sus scrofa* (pig)), P09115 (rabbit) and O97943 (*Camelus dromedarius* (camel));  $\beta$ -caseins: NP 851351 (*Bos taurus* (bovine)), NP 058816 (*Rattus norvegicus* (rat)), NP 001882 (*Homo sapiens* (human)), NP 034102 (*Mus musculus* (mouse)), CAB39313 (*Capra hircus* (goat)), CAA06535 (*Bubalus bubalis* (water buffalo)), CAA38718 (*Sus scrofa* (pig)), BAA95931 (*Canis familiaris* (dog)), and CAA34502 (*Ovis aries* (sheep));  $\kappa$ -caseins: NP 776719 (*Bos taurus* (bovine)), NP 113750 (*Rattus norvegicus* (rat)), NP 031812 (*Mus musculus* (mouse)), NP 005203 (*Homo sapiens* (human)) and AAM12027 (*Capra hircus* (goat)).

As used herein the term "N terminus portion" refers to M amino acids of  $\alpha$ S1 casein derived from the first 60 amino acids of  $\alpha$ S1 casein, wherein M is

any of the integers between 2 and 60 (including the integers 2 and 60). Preferably, the term refers to the first M amino acids of  $\alpha$ S1 casein.

The peptides of the invention can be obtained by extraction from milk as previously described, or by solid phase peptide synthesis, which is a standard  
5 method known to the man skilled in the art. Purification of the peptides of the invention is performed by standard techniques, known to the man skilled in the art, such as high performance liquid chromatography (HPLC), diafiltration on rigid cellulose membranes (Millipore) and gel filtration. Milk casein fragmentation to obtain the peptides of the invention may be effected using  
10 various enzymatic and/or chemical means, as described hereinbelow.

As is further detailed hereinunder and exemplified in the Examples section that follows, the peptides of the present invention have a variety of therapeutic effects. In the Examples section there are provided numerous assays with which one of ordinary skills in the art can test a specific peptide  
15 designed in accordance with the teachings of the present invention for a specific therapeutic effect. Any of the peptides described herein can be administered *per se* or be formulated into a pharmaceutical composition which can be used for treating or preventing a disease. Such a composition includes as an active ingredient any of the peptides described herein and a  
20 pharmaceutically acceptable carrier.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the peptides described herein, with other chemical components such as pharmaceutically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an  
25 organism.

Hereinafter, the term "pharmaceutically acceptable carrier" refers to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. Examples, without limitations, of carriers are: propylene glycol,  
30 saline, emulsions and mixtures of organic solvents with water. Herein the term

"excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, transdermal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active peptides into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the peptides of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer with or without organic solvents such as propylene glycol, polyethylene glycol. For transmucosal administration, penetrants are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the peptides can be formulated readily by combining the active peptides with pharmaceutically acceptable carriers well known in the art. Such carriers enable the peptides of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active ingredient doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active peptides may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral

administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

5 For administration by inhalation, the peptides according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-  
10 tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The peptides described herein may be formulated for parenteral  
15 administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or  
20 dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active peptides may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty  
25 oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the  
30 peptides to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The peptides of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g.,  
5 conventional suppository bases such as cocoa butter or other glycerides.

The pharmaceutical compositions herein described may also comprise suitable solid of gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin and polymers  
10 such as polyethylene glycols.

Persons ordinarily skilled in the art can easily determine optimum dosages and dosing methodology for any of the peptides of the invention.

For any peptide used in accordance with the teachings of the present invention, a therapeutically effective amount, also referred to as a  
15 therapeutically effective dose, which can be estimated initially from cell culture assays or *in vivo* animal assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the  $IC_{50}$  or the  $IC_{100}$  as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Initial dosages can also be  
20 estimated from *in vivo* data. Using these initial guidelines one having ordinary skill in the art could determine an effective dosage in humans.

Moreover, toxicity and therapeutic efficacy of the peptides described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the  $LD_{50}$  and the  $ED_{50}$ . The dose  
25 ratio between toxic and therapeutic effect is the therapeutic index and can be expressed as the ratio between  $LD_{50}$  and  $ED_{50}$ . Peptides which exhibit high therapeutic indices are preferred. The data obtained from these cell cultures assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of such peptides lies preferably within a  
30 range of circulating concentrations that include the  $ED_{50}$  with little or no

toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, In: The Pharmacological Basis of Therapeutics, chapter 1, page 1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active ingredient which are sufficient to maintain therapeutic effect. Usual patient dosages for oral administration range from about 1-1000 mg/kg/administration, commonly from about 10-500 mg/kg/administration, preferably from about 20-300 mg/kg/administration and most preferably from about 50-200 mg/kg/administration. In some cases, therapeutically effective serum levels will be achieved by administering multiple doses each day. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow release composition, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the



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manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a peptide of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment or prevention of an indicated condition or induction of a desired event. Suitable indicia on the label may include treatment and/or prevention of an autoimmune disease or condition, viral disease, viral infection, bacterial infection, hematological disease, hematological deficiencies, thrombocytopenia, pancytopenia, granulocytopenia, an erythropoietin treatable condition, a thrombopoietin treatable condition, hyperlipidemia, hypercholesterolemia, glucosuria, hyperglycemia, diabetes, AIDS, infection with HIV-1, a coronavirus or SARS infection, helper T-cell disorders, dendrite cell deficiencies, macrophage deficiencies, hematopoietic stem cell disorders including platelet, lymphocyte, plasma cell and neutrophil disorders, hematopoietic stem cell proliferation, hematopoietic stem cell proliferation and differentiation, pre-leukemic conditions, leukemic conditions, immune system disorders resulting from chemotherapy or radiation therapy, and human immune system disorders resulting from treatment of diseases of immune deficiency.

The pharmaceutical compositions according to the invention may be useful in maintaining and/or restoring blood system constituents, in balancing blood cell counts, in balancing levels of metabolites in the blood including sugar, cholesterol, calcium, uric acid, urea and enzymes such as alkaline phosphatase. Further, the pharmaceutical compositions of the invention may be useful in inducing blood cell proliferation, modulating white and/or red blood cell counts, particularly increasing white and/or red blood cell counts, elevating haemoglobin blood level and in modulating platelet counts.

The term "balancing" as used herein with relation to levels of certain physiological parameters, means changing the levels of referred parameters and bringing them closer to normal values. As used herein, the term "modulating", with regard to physiological processes such as blood cell formation, is defined  
5 as effecting a change in the quality and/or amount of said processes, including, but not limited to, increasing and decreasing frequency, character, duration, outcome, magnitude, cyclic nature, and the like. Examples of such modulation are  $\alpha$ S1-casein and  $\beta$ -casein's enhancement of megakaryocyte proliferation, dendritic cell proliferation, and effect of G-CSF on CFU-GM colony growth, as  
10 described hereinbelow. It will be appreciated that, in the context of a preferred embodiment of the present invention, such "balancing" and/or "modulating" of physiological and metabolic parameters comprises modification of biological responses, and as such, peptides derived from  $\alpha$ -,  $\beta$ - and  $\kappa$ -casein, alone or in combination therewith, can be "biological response modifiers".

15 The term "normal values" as used herein with relation to physiological parameters, means values which are in the range of values of healthy humans or animals. However, it will be appreciated that nominally "healthy" subjects, having physiological parameters within or close to the ranges of values conventionally considered normal, can benefit from further "balancing" and  
20 "modulation" of such physiological parameters, towards the optimization thereof.

In specifically preferred embodiments, the peptides of the invention are use to treat or prevent blood disease or conditions, and balance counts of red blood cells, white blood cells, platelets and haemoglobin level. The  
25 pharmaceutical compositions of the invention may be used for activating blood cell proliferation.

In addition, the pharmaceutical compositions may be used for the treatment and/or prevention of hemopoietic stem cell disorders, including platelet, lymphocyte, plasma cell, dendritic cell and neutrophil disorders, as

well as deficiency and malfunction in pre-leukemic and leukemic conditions and thrombocytopenia.

Further, the pharmaceutical compositions may be used for modulating blood cell formation, including the treatment and/or prevention of cell proliferative diseases. In this connection, it is worth noting that the pharmaceutical compositions of the invention are advantageous in the stimulation of the immune response during chemotherapy or radiation treatments, in alleviating the negative effects, reducing chemotherapy and irradiation-induced vomiting and promoting a faster recovery.

Still further, the pharmaceutical compositions of the invention may be used for the stimulation of human immune response during treatment of diseases associated with immune deficiency, for example HIV and autoimmune diseases.

The compositions of the invention may also be intended for veterinary use.

The pharmaceutical compositions of the invention may be used in the treatment and/or prevention of, for example, disorders involving abnormal levels of blood cells, disorders involving hematopoietic stem cells production and differentiation, treatment of erythrocyte, platelet, lymphocyte, dendritic cell, macrophage and/or neutrophil disorders, for the treatment of pre-leukemic and leukemic conditions and for the treatment of thrombocytopenia. The pharmaceutical compositions of the invention may also be used in the treatment of cell proliferative diseases and diseases involving immune deficiency, such as HIV, and of autoimmune diseases. Further, the pharmaceutical compositions of the invention may be used for modulating the immune response during chemotherapy or radiation treatments, for example for reducing chemotherapy-associated vomiting.

While reducing the present invention to practice, it was surprisingly observed that the peptides of the invention exert a synergistic effect on human hematopoietic stem cell proliferation and differentiation with addition of other

hematopoietic growth factors. Of notable significance was the potentiation of erythropoietin-mediated stimulation of erythroid colony formation, the potentiation of G-CSF-mediated stimulation of granulocyte macrophage colony formation (CFU-GM) in bone marrow cells, and the dose-dependent enhancement of thrombopoietin (TPO) induction of megakaryocyte proliferation by peptides of the present invention. G-CSF is currently used for mobilization of bone marrow hematopoietic progenitor cells in donors, as a component of a wide variety of leukemia and cancer treatments (see, for example, U.S. Patent Nos: 6,624,154 to Benoit et al. and 6,214,863 to Bissery et al) and as a component of cell growth media for stem and progenitor cell manipulation (see, for example, US Patent No: 6,548,299 to Pykett et al). Recombinant human (rh) G-CSF, marketed as Neupogen (Filgrastim, Amgen Inc., USA) has been approved for medical use for indications relating to neutropenia and granulocytopenia, such as AIDS leukopenia and febrile neutropenia, respiratory and other infection (Kolls et al, Resp. Res. 2000; 2:9-11) and in chemotherapy protocols for non-myeloid malignancies. Recombinant human (rh) EPO is currently an approved therapy for indications such as renal anemia, anemia of prematurity, cancer- and AIDS-associated anemia, and for pre-elective surgical treatment (Sowade, B et al. Int J Mol Med 1998;1:305).

Thus, in one preferred embodiment, a blood disease or condition such as thrombocytopenia, pancytopenia, granulocytopenia, an erythropoietin treatable condition, a thrombopoietin treatable condition, or a G-CSF treatable condition is treated by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof.

Further according to the present invention there is provided a method of augmenting the effect of erythropoietin, thrombopoietin, or G-CSF, the method is effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a

combination thereof. In one preferred embodiment, the method further comprises administering a blood cell stimulating factor such as erythropoietin, thrombopoietin, and G-CSF.

Thrombopoietin is an early acting cytokine with important multilineage effects: TPO alone, or in combination with other early acting cytokines, can (i) promote viability and suppress apoptosis in progenitor cells; (ii) regulate hematopoietic stem cell production and function; (iii) trigger cell division of dormant multipotent cells; (iv) induce multilineage differentiation and (v) enhance formation of multilineage colonies containing granulocytes, erythrocytes, macrophages, and megakaryocytes (MK, CFU-GEMM). Moreover, TPO stimulates the production of more limited progenitors for granulocyte/monocyte, megakaryocyte and erythroid colonies, stimulates adhesion of primitive human bone marrow and megakaryocytic cells to fibronectin and fibrinogen. G-CSF is similar in action, but is specific for cells of granulocyte lineage, while EPO stimulates development of red blood cells and red blood cell progenitors. Thus, TPO, EPO and G-CSF are important cytokines for clinical hematologists/transplanters: for the mobilization, amplification and ex vivo expansion of stem cells and committed precursor cells for autologous and allogeneic transplantation. In addition, administration of TPO and G-CSF to healthy platelet donors has been employed to enhance pheresis yields. However, clinical application of TPO, EPO and G-CSF therapy is complicated by, among other considerations, relatively high costs of the recombinant human cytokine rhTPO, EPO and G-CSF and the potential antigenicity of TPO, EPO and G-CSF with repeated administration.

Combined treatment with such blood cell stimulating factor as TPO, EPO and G-CSF, and the peptide of the present invention, either together in a pharmaceutical composition comprising both, or separately, can provide inexpensive, proven non-toxic augmentation of the cytokines effects on target cell proliferation and function. In such a combination, the peptide of the present invention may be applied to the treatment of, in addition to the

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abovementioned conditions, disorders such as myelodysplastic syndrome (MDS), non-myeloid malignancies, aplastic anemia and complications of liver failure. Pre-treatment of platelet donors with the peptide of the present invention, alone or in combination with TPO and G-CSF, may even further  
5 enhance the efficiency of pheresis yields.

Thus, according to the present invention there is provided a method of preventing or treating a blood disease or condition, such as a thrombopoietin treatable condition, an erythropoietin treatable condition, and a G-CSF treatable condition, the method is effected by administering to a subject in need thereof a  
10 therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof.

Further according to the present invention there is provided a method of augmenting the effect of thrombopoietin, erythropoietin, and G-CSF, the method is effected by administering to a subject in need thereof a  
15 therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof.

Further according to the present invention there is provided a method of modulating blood cell formation, the method is effected by administering to a subject in need thereof an effective amount of a pharmaceutical composition  
20 comprising effective amounts of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof alone, or in combination with blood cell stimulating factors such as thrombopoietin, erythropoietin, and G-CSF, as described hereinabove.

In one preferred embodiment, modulating blood cell formation includes  
25 inducing hematopoiesis, inducing hematopoietic stem cell proliferation, inducing hematopoietic stem cell proliferation and differentiation, inducing megakaryocytopoiesis, inducing erythropoiesis, inducing leukocytopoiesis, inducing thrombopoiesis, inducing plasma cell proliferation, inducing dendritic cell proliferation and inducing macrophage proliferation. In a yet more  
30 preferred embodiment, the peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a

combination thereof is a synthetic peptide, alone or in combination with other, non-identical peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, as described hereinabove.

Further according to the present invention there is provided a  
5 pharmaceutical composition for treating a blood disease or condition, such as a thrombopoietin treatable condition, an erythropoietin treatable condition, and a G-CSF treatable condition, the pharmaceutical composition comprising, as an active ingredient a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a pharmaceutically acceptable carrier.

10 Further according to the present invention there is provided a pharmaceutical composition for augmenting the effect of a blood cell stimulating factor, such as thrombopoietin, erythropoietin and G-CSF, the pharmaceutical composition comprising, as an active ingredient a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a  
15 pharmaceutically acceptable carrier.

Further according to the present invention there is provided a pharmaceutical composition for modulating blood cell formation, the pharmaceutical composition comprising, as active ingredients a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof alone, or in combination  
20 with blood cell stimulating factors such as thrombopoietin, erythropoietin, and G-CSF, and a pharmaceutically acceptable carrier.

In preferred embodiments, modulating blood cell formation includes inducing hematopoiesis, inducing hematopoietic stem cell proliferation, inducing hematopoietic stem cell proliferation and differentiation, inducing  
25 megakaryocytopoiesis, inducing erythropoiesis, inducing leukocytopoiesis, inducing thrombopoiesis, inducing plasma cell proliferation, inducing dendritic cell proliferation, and inducing macrophage proliferation. Methods of monitoring the modulation of blood cell formation, both *in vivo* and *in vitro*, are well known in the art, and are described in detail in the Examples section  
30 below.

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Mobilization of stem cells from the bone marrow to the peripheral circulation is required in a number of medical protocols. For example, in preparation for chemotherapeutic or radiation treatment of proliferative disorders such as cancer, the patients stem cells are first mobilized from the bone marrow, usually via G-CSF, and collected for later reconstitution. Similarly, in heterologous stem cell reconstitution, the donor is treated with factors to mobilize stem cells to the peripheral circulation prior pheresis. Methods of mobilization of stem cells to the peripheral circulation are well known in the art (see, for example, US Patent Application No: 6,162,427 to Baumann et al., incorporated herein by reference).

While reducing the present invention to practice, it was uncovered that peptides derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof enhanced and stimulated proliferation of hematopoietic cells *in vivo* and *in vitro*. Thus, according to the present invention there is provided a method of enhancing peripheral stem cell mobilization, the method is effected by administering to a subject in need thereof an effective amount of a pharmaceutical composition comprising effective amounts of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof alone, or in combination with blood cell stimulating factors such as thrombopoietin, erythropoietin, and G-CSF, as described hereinabove.

Further according to the present invention there is provided a pharmaceutical composition for treating or preventing an indication selected from the group consisting of hematological disease, hematological deficiencies, thrombocytopenia, pancytopenia, granulocytopenia, dendritic cell deficiencies, macrophage deficiencies, hematopoietic stem cell disorders including platelet, lymphocyte, plasma cell and neutrophil disorders, pre-leukemic conditions, leukemic conditions, myelodysplastic syndrome, non-myeloid malignancies, aplastic anemia and bone marrow insufficiency, the pharmaceutical composition comprising, as active ingredients, a blood cell stimulating factor such as thrombopoietin, erythropoietin or G-CSF and a peptide derived from an



$\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a pharmaceutically acceptable carrier.

Further according to the present invention there is provided a pharmaceutical composition comprising a blood cell stimulating factor and a  
5 purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-33 and a pharmaceutically acceptable carrier. In one preferred embodiment, the blood cell stimulating factor is TPO, EPO or G-CSF.

Further according to the present invention there is provided a method of  
10 enhancing colonization of donated blood stem cells in a myeloablated recipient, the method is effected by treating a donor of the donated blood stem cells with a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof prior to implanting the donated blood stem cells in the recipient.

Further according to the present invention there is provided a method of  
15 enhancing colonization of donated blood stem cells in a myeloablated recipient, the method is effected by treating the donated blood stem cells with a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof prior to implanting the donated blood stem cells in the recipient.

Further according to the present invention there is provided a method of  
20 enhancing colonization of blood stem cells in a myeloablated recipient, the method is effected by treating the blood stem cells with a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof prior to implanting the blood stem cells in the recipient. In one preferred embodiment, the blood stem cell donor, or blood stem cells, or donated blood stem cells are further treated with a  
25 blood cell stimulating factor such as thrombopoietin, erythropoietin or G-CSF, prior to donation and implanting the blood stem cells in the recipient. In another preferred embodiment, the peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof is in combination with other, identical or non-identical peptide or peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein.

Further according to the present invention there is provided a pharmaceutical composition for enhancing colonization of donated blood stem cells in a myeloablated recipient, the pharmaceutical composition comprising, as active ingredients, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a pharmaceutically acceptable carrier.

Further according to the present invention there is provided a pharmaceutical composition for enhancing colonization of blood stem cells in a myeloablated recipient, the pharmaceutical composition comprising, as active ingredients, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a pharmaceutically acceptable carrier.

In one preferred embodiment, the pharmaceutical composition further comprises a blood cell stimulating factor such as thrombopoietin, erythropoietin or G-CSF. In another preferred embodiment, the peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof is in combination with a peptide or peptides derived from identical or non-identical  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein.

The invention further relates to anti-bacterial pharmaceutical compositions comprising as active ingredient at least one peptide of the invention and to the use of the peptides of the invention as anti-bacterial agents.

As detailed in the Examples section hereinbelow, peptides of the invention, and pharmaceutical compositions comprising as an active ingredient a peptide of the invention, can be used in the treatment and prevention of blood cell disorders, cell proliferative diseases, diseases involving immune deficiency and autoimmune diseases.

Thus, according to the present invention there is provided a method of preventing or treating an autoimmune or infectious disease or condition, the method is effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof.

In one embodiment, the autoimmune or infectious disease or condition is a viral disease, a viral infection, AIDS and infection by HIV.

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Further according to the present invention there is provided a method of preventing or treating thrombocytopenia, the method is effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof.

5 Further according to the present invention there is provided a method of preventing or treating pancytopenia, the method is effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof.

10 Further according to the present invention there is provided a method of preventing or treating granulocytopenia, the method is effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof.

While reducing the present invention to practice, it was surprisingly uncovered that administration of peptides derived from an N terminus portion  
15 of  $\alpha$ S1 casein effectively prevented the onset of diabetic symptoms in genetically predisposed NOD mice, and balanced blood chemistry values in both human subjects having familial hypercholesterolemia and triglyceridemia, and in animal models. Thus, according to the present invention there is provided a method of preventing or treating a metabolic disease or condition,  
20 the method is effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof. In preferred embodiments, the metabolic disease or condition is non-insulin dependent diabetes mellitus, insulin-dependent diabetes mellitus, glucosuria, hyperglycemia, hyperlipidemia, and/or  
25 hypercholesterolemia.

As used herein, the term "metabolic disease or condition" is defined as a deviation or deviations from homeostatic balance of metabolites in the body, as expressed by abnormal levels of certain physiological parameters measurable in the body. Such physiological parameters can be, for example, hormone levels,  
30 electrolyte levels, blood glucose levels, enzyme levels, and the like.

Further according to the present invention there is provided a method of preventing or treating conditions associated with myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT), the method is effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an N terminus portion of  $\alpha$ S1 casein, alone or in combination with a blood cell stimulating factor such as thrombopoietin, erythropoietin or G-CSF.

Further according to the present invention there is provided a pharmaceutical composition for preventing or treating an autoimmune or infectious disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a pharmaceutically acceptable carrier. In preferred embodiments, the disease or condition is a viral disease, a viral infection, AIDS, and/or infection by HIV. In further preferred embodiments, the peptide of the invention is administered as an adjunct therapy, in combination with additional treatment against viral and other infection, or to prevent onset, or reduce the severity of disease symptoms following viral infection, as in HIV and AIDS therapy.

Further according to the present invention there is provided a pharmaceutical composition for preventing or treating a metabolic disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a pharmaceutically acceptable carrier. In preferred embodiments, the metabolic disease or condition is non-insulin dependent diabetes mellitus, insulin-dependent diabetes mellitus, glucosuria, hyperglycemia, hyperlipidemia, and/or hypercholesterolemia.

Further according to the present invention there is provided a method of preventing or treating conditions associated with myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood

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stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT), the method is effected by administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, alone or in combination with a blood cell stimulating factor such as thrombopoietin, erythropoietin or G-CSF.

Further according to the present invention there is provided a pharmaceutical composition for preventing or treating an autoimmune or infectious disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, alone or in combination with other identical or non-identical  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein peptides, and a pharmaceutically acceptable carrier. In preferred embodiments, the disease or condition is a viral disease, a viral infection, AIDS, and/or infection by HIV. In further preferred embodiments, the peptide of the invention is administered as an adjunct therapy, in combination with additional treatment against viral and other infection, or to prevent onset, or reduce the severity of disease symptoms following viral infection, as in HIV and AIDS therapy.

Further according to the present invention there is provided a pharmaceutical composition for preventing or treating a metabolic disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a pharmaceutically acceptable carrier. In preferred embodiments, the metabolic disease or condition is non-insulin dependent diabetes mellitus, insulin-dependent diabetes mellitus, glucosuria, hyperglycemia, hyperlipidemia, and/or hypercholesterolemia.

Further according to the present invention there is provided a pharmaceutical composition for preventing or treating conditions associated with myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT), the pharmaceutical composition

comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof and a pharmaceutically acceptable carrier.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for  
5 preventing or treating an autoimmune disease.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating a viral disease.

Further according to the present invention there is disclosed the use of a  
10 peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing viral infection.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing hematopoiesis.

15 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing hematopoietic stem cells proliferation.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing  
20 hematopoietic stem cells proliferation and differentiation.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing megakaryocytopoiesis.

Further according to the present invention there is disclosed the use of a  
25 peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing erythropoiesis.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing leukocytopoiesis.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing thrombocytopoiesis.

5 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing plasma cell proliferation.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing dendritic cell proliferation.

10 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for inducing macrophage proliferation.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating thrombocytopenia.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating pancytopenia.

20 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating granulocytopenia.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating hyperlipidemia.

25 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating cholesteremia.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating glucosuria.

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Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating diabetes.

5 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating AIDS.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating infection by HIV.

10 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for preventing or treating conditions associated with myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation  
15 (BMT).

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for treating a thrombopoietin treatable condition.

20 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for augmenting the effect of thrombopoietin.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for enhancing peripheral stem cell mobilization.

25 Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for enhancing colonization of donated blood stem cells in a myeloablated recipient.

Further according to the present invention there is disclosed the use of a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof for  
30 enhancing colonization of blood stem cells in a myeloablated recipient.



Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating an autoimmune disease.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, and a pharmaceutically acceptable carrier for preventing or treating a viral disease.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof casein, and a pharmaceutically acceptable carrier for preventing or treating a viral infection.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing hematopoiesis.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing hematopoietic stem cell proliferation.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing hematopoietic stem cells proliferation and differentiation.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide

derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing megakaryocytopoiesis,

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
5 derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing erythropoiesis.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
10 derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing leukocytopoiesis.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing thrombocytopoiesis.

15 Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing plasma cell proliferation,

Further according to the present invention there is disclosed the use of a  
20 pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing dendritic cell proliferation.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
25 derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for inducing macrophage proliferation.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a

pharmaceutically acceptable carrier for preventing or treating thrombocytopenia.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
5 derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating pancytopenia.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
10 derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating granulocytopenia.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating hyperlipidemia.

15 Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating cholesteremia.

Further according to the present invention there is disclosed the use of a  
20 pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating glucosuria.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
25 derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating diabetes.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide  
30 derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating AIDS.

Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating infection by HIV.

5 Further according to the present invention there is disclosed the use of a pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or a combination thereof, and a pharmaceutically acceptable carrier for preventing or treating conditions associated with myeloablative doses of chemoradiotherapy supported by  
10 autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT).

Further according to the present invention there is provided a purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs:1-33.

15 Further according to the present invention there is provided a pharmaceutical composition comprising a purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs:1-33 and a pharmaceutically acceptable carrier.

The invention further relates to methods of treatment comprising the  
20 administration of, and pharmaceutical compositions comprising, combinations of peptides derived from  $\alpha$ -  $\beta$ - and  $\kappa$ -casein. While reducing the present invention to practice, it was uncovered that combinations of peptides derived from  $\alpha$ S1 casein and peptides derived from  $\beta$ -casein were more effective in enhancing leukocyte proliferation following bone marrow reconstitution in  
25 mice than the individual peptides administered alone (see Fig. 25). In one embodiment, the combination of peptides comprises a mixture of peptides. In a preferred embodiment, the combination of peptides comprises chimeric peptides covalently linked as described hereinabove.

The invention further relates to anti-viral pharmaceutical compositions comprising as active ingredient at least one peptide of the invention and to the use of the peptides of the invention as anti-viral agents. While reducing the present invention to practice, it was uncovered that peptides derived from natural casein have efficient immuno-modulatory activity that is completely free of any demonstrable side effects.

As described in detail in the Examples section hereinbelow, peptides derived from natural casein are capable of stimulating proliferation of various types of blood stem cells and can effectively enhance reconstitution of white blood cells and platelets even in patients who are completely resistant to platelet transfusion. Peptides derived from natural casein are effective in patients who are completely resistant to other modalities known to potentially enhance platelet reconstitution (including rhIL-3 and rhIL-6). Peptides derived from natural casein are an efficient immunomodulator capable of enhancing hematopoietic processes of different blood stem cells with a powerful effect on White Blood Cells (WBC), platelet reconstitution and stimulation of NK activity.

Thus, according to a further aspect of the present invention there is provided a method of treating or preventing a condition associated with a SARS infective agent, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from an N terminus portion of  $\alpha$ S1 casein.

Further according to the present invention there is provided a pharmaceutical composition for preventing or treating a condition associated with a SARS infective agent, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from an N terminus portion of  $\alpha$ S1 casein and a pharmaceutically acceptable carrier. In a preferred embodiment the SARS infective agent is a coronavirus. In a most preferred embodiment the coronavirus is SARS-CoV.

It will be appreciated by one of ordinary skill in the art, that the efficacy of compositions of peptides derived from natural casein for prevention and/or treatment of conditions associated with SARS infective agent can be evaluated both in vitro and in clinical trials. Recently, Rota *et al.* (Scienceexpress, 1 May 2003, see [www.sciencexpress.org](http://www.sciencexpress.org)) reported the characterization of the SARS-CoV virus, and successful in-vitro growth and isolation of SARS-CoV in Vero cells. Thus, for example, as described hereinbelow for HIV-1, Vero cells can be exposed to compositions of peptides derived from natural casein both prior to and following exposure to a SARS infective agent, and the levels of infection can be determined, for example, via measurement of viral specific transcripts, protein products or virion production using methods well known in the art.

As detailed hereinabove, the  $\alpha$ S2,  $\beta$ , and  $\kappa$ -fractions of casein have been shown to contain peptides having advantageous biological properties. It will be appreciated that combinations of peptides derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, and other identical or non-identical casein derived peptides (such as  $\alpha$ S2-,  $\beta$ - and  $\kappa$ -casein) can have a synergistic effect on the modulation and enhancement of hematopoietic, immunological, EPO-, TPO-, G-CSF-mediated, anti-viral and other processes for which peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein have been shown herein to be effective. Thus, further according to the present invention there is provided a pharmaceutical composition comprising peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in combination with other identical or non-identical peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, wherein said combination is a mixture of peptides or a chimeric peptide.

While reducing the present invention to practice, a low-temperature method for processing casein hydrolysate at low temperatures was conceived. This novel method for the inactivation and removal of the protease following digestion of the casein, is superior in rapidity and ease, and without the undesirable disadvantages of traditional methods using heat inactivation. By replacing the high ( $>75^{\circ}\text{C}$ ) heat inactivation step with cooling and

alkalinization, effective and absolute inactivation of the proteases, with no danger to the peptides, was achieved.

Thus, according to a further aspect of the present invention there is provided a method of low-temperature processing of casein proteolytic hydrolysate, the method is effected by obtaining a casein proteolytic hydrolysate comprising proteolytic enzymes, cooling the casein proteolytic hydrolysate so as to inactivate the proteolytic enzymes, adjusting the pH of the casein protein hydrolysate to an acid pH, filtering the acidic casein protein hydrolysate and collecting the filtrate. Methods for batch cooling of the casein hydrolysate, following proteolytic digestion, are well known in the art (see, for example, industrial fermentor and bioreactor temperature control systems from BioGenTek, New Delhi, India) and in the dairy products industry (suitable heat exchange systems for large and small volume applications are widely available commercially).

The filtrate is then further acidified so as to precipitate the proteins derived from natural casein, separated and collected, and then the pH of the precipitate is adjusted to an alkaline pH with a base such as NaOH, so as to irreversibly inactivate the proteolytic enzymes. Following inactivation of the proteolytic enzymes, the pH of the precipitate is readjusted with acid, such as HCl, to pH 7-9, thereby processing the casein protein hydrolysate at low temperature. In a preferred embodiment, the casein hydrolysate is cooled to about 10°C, most preferably to 8-10 °C. Temperature is maintained at 10 °C by addition of cold TCA, and centrifugation at a temperature less than 10°C.

In a further embodiment, the pH is adjusted to acid pH by addition of acid to 2% (w/v) acid, and further acidifying the filtrate is effected by additional addition of acid to about 10% (w/v) acid. In a preferred embodiment, the alkaline pH of the precipitate is adjusted with a base to at least pH 9, preferably pH 10, most preferably pH 13. In preferred embodiment, alkaline pH is maintained for greater than 15 minutes, more preferred for greater than 30 minutes, and in a most preferred embodiment greater than 1

hour. Monitoring of the residual proteolytic activity following cooling and alkaline treatment, can be used to determine the optimal range of alkaline treatment.

As used herein, the term "about" is defined as the range comprising from 20% greater than to 20% less than the indicated value. Thus, the phrase "about 10°C", as used herein, includes the range of temperatures from 8°C to 12°C. Similarly, the phrase "about 10% (w/v) acid" includes the range of acid content from 8% w/v to 12% w/v.

The present invention successfully addresses the shortcomings of the presently known configurations by providing peptides for the treatment of human disease, which peptides are derived from an  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, alone or in combination with other identical or non-identical peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein, and possess no detectable toxicity and high therapeutic efficacy.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

### EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

### MATERIALS AND EXPERIMENTAL METHODS

**Preparation of peptides derived from natural casein:** The casein fraction of cow's milk was isolated as described by Hipp *et al.* (1952), *ibid.*, or provided as commercial casein, and subjected to exhaustive proteolytic digestion with chymosin (also known as rennin) (20 ng per ml) at 30 °C. Upon



completion of the reaction, the solution was heated to inactivate the enzyme, and the digest was precipitated as paracaseinate by acidification with an organic acid, acetic or trichloroacetic acid. Paracaseinate was separated by centrifugation, and the supernatant fraction, containing the peptide fragments of interest, was re-precipitated as caseicidin by higher acid concentrations. The resulting caseicidin, following re-suspension, dialysis and neutralization was lyophilized. The resulting powdered preparation was assayed for biological activity as described below, and separated by HPLC for peptide analysis.

Alternatively, the caseicidin can be prepared by cooling and alkaline treatment. Following digestion of the casein, the reaction mixture was cooled immediately to below 10°C and cold TCA (Tri-chloro acetic acid) was added to obtain a 2% TCA solution. The solution was separated by centrifugation at 1370Xg, at a temperature less than 10°C.

The supernatant was removed and filtered. Additional cold TCA was added to obtain a 10%-12.5% TCA solution. The solution was centrifuged at 1370xg, at a temperature below 10°C. The precipitate was removed and dissolved in H<sub>2</sub>O and made alkaline by a strong base, such as, for example, NaOH, to increase the pH of the hydrolysate to pH 9-13. The solution was maintained at basic pH between 15 min to 1 hour. Subsequently, the solution was acidified to pH 7- 9 by addition of an acid such as HCl. The resultant mixture of peptides was further fractionated and purified by gel filtration on a dextran column (such as Sephadex), as described herein, or by diafiltration on a series of rigid membranes, for example, using a first diafiltration apparatus with a 10 kDa cutoff, and a second diafiltration apparatus with a 3 kDa cutoff (Millipore, Billerica, MA, USA).

**HPLC analysis of peptides derived from natural casein:** Peptides derived from natural casein as described above were analyzed by HPLC in two stages. Initially, the lyophilized casein digests were separated using a C 18 reversed phase with a 0.1 % water trifluoroacetic acid (w/w)-acetonitrile gradient. Detection was according to UV absorption at 214 nm. Following this

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the samples were analyzed by HPLC-Mass Spectroscopy (MS) equipped with an electrospray source. Mass calculations represent the mass of the ionized peptide samples, as derived from the retention times. Following separation, the amino acid composition of the peptides was determined with a gas-phase  
5 microsequencer (Applied Biosystems 470A).

Analysis of some preparations of peptides derived from natural casein produced the following results: Eight peptide peaks were typically observed of which 3 were major peaks having Rt values of 17.79, 19.7, 23.02 and 5 were minor peaks having Rt values of 12.68, 14.96, 16.50, 21.9 and 25.1, which Rt  
10 values represent molecular mass of 2764, 6788, 1880, 2616, 3217, 2333, 6708 and 6676 Da, respectively. At Rt of 17.79 (corresponding to 2,764 Da) a major peak of a peptide of 23 amino acids representing amino acids 1-23 of  $\alpha$ S1 casein, having the sequence RPKHPIKHQGLPQEVLENENLLRF (SEQ ID NO:22, see McSweeney et al., 1993, *ibid.*, for the complete sequence of  $\alpha$ S1  
15 casein). Other peptides were from positions 208-224 of  $\beta$ -like casein precursor, positions 16-37 of  $\alpha$ S1 casein and positions 197-222 of  $\alpha$ S2-like casein precursor. Other peptides were also present. Peptides derived from natural casein were further analysed with HPLC-MS (C-18 resin) and sequenced using MS/MS and Edman degradation. The column used was Vydac C-18, and the  
20 elution was carried out with a gradient starting with 2% CH<sub>3</sub>CN, 0.1% TFA and continues by increasing modifier (2% H<sub>2</sub>O, 0.1% TFA in CH<sub>3</sub>CN) up to 80% at 80min. Mass Spectrometry was carried out with Qtof2 (Micromass, England), using a nanospray attachment.

Edman degradation was carried out using a Perkin Elmer (Applied Biosystems Division) 492 (procise) Microsequencer system. Further HPLC-MS was also carried out using a C-12 resin. Analysis of peptides derived from natural casein revealed three major components:

5        i) a peptide representing an N-terminal portion of  $\alpha$ S1 casein, amino acid coordinates 1-23 of the processed peptide (SEQ ID No: 22). Molecular mass is 2764 daltons.

         ii) a peptide representing amino acid coordinates 193-209 of  $\beta$  casein (SEQ ID No. 27). Molecular mass is 1880 daltons.

10       iii) a peptide representing amino acid coordinates 106-169 of  $\kappa$  casein (SEQ ID No. 29). Molecular mass is 6708 daltons. The  $\kappa$  casein was found in two forms: a phosphorylated form, and an un-phosphorylated form. The molecular mass of the phosphorylated peptide is 6789 daltons. Further there was identified a known variant of  $\kappa$  casein, whose molecular mass is 6676 Da  
15 (non-phosphorylated). Three minor components were identified:

         i) a peptide representing an N-terminal portion of  $\alpha$ S1 casein, amino acid coordinates 1-22 of the processed peptide (SEQ ID No: 21). Molecular mass is 2616 daltons.

         ii) a peptide representing amino acid coordinates 165-199 of  $\alpha$ S1 casein  
20 (SEQ ID No. 31). Molecular mass is 3918 daltons.

         iii) a peptide representing amino acid coordinates 182-207 of  $\alpha$ S2 casein (SEQ ID No. 32). Molecular mass is 3217 daltons.

         iv) a peptide representing amino acid coordinates 189-207 of  $\alpha$ S2 casein (SEQ ID No. 33). Molecular mass is 2333 daltons.

25       Minor peptides representing portions of the N-terminal of  $\beta$  casein, and other portions of bovine casein were also detected.

***Gel filtration of peptides derived from natural casein:***

Peptides derived from natural casein, prepared as described hereinabove, were separated according to molecular mass by gel filtration using Superdex75

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Gel filtration column by Pharmacia. The elution buffer used for the preparative separation was  $\text{NH}_4\text{HCO}_3$ , pH = 8. The following purified fractions were obtained: a peptide representing amino acid positions 1-23 of the N-terminus of  $\alpha\text{S1}$  casein (SEQ ID No. 22), and a second peptide representing amino acid positions 106-169 of  $\kappa$ -casein (SEQ ID No. 29). Without wishing to be limited by a single hypothesis, one explanation for the apparent discrepancy between the analyses of the peptides derived from natural casein by the HPLC, HPLC MS and gel filtration methods is the tendency of gel filtration to retard specific components of a mixture of peptides.

**Synthetic peptides derived from casein:** Peptides of increasing lengths corresponding to the N-terminal 2-26 amino acids of  $\alpha\text{S1}$  casein were synthesized by NoVetide Ltd., Haifa, Israel, with purity of >95 % (HPLC). Quality Control included: HPLC, Mass Spectrometry (EI), Amino acid analysis and Peptide Content. Table 3 below provides the sequence of these peptides:

TABLE 3

Identification	Sequence (N terminus - C terminus)	No. of amino acids	SEQ ID NO:
74	RP	2	1
1P	RPK	3	2
2P	RPKH	4	3
3P	RPKHP	5	4
4P	RPKHPI	6	5
5P	RPKHPIK	7	6
Y	RPKHPIKH	8	7
X	RPKHPIKHQ	9	8
1a	RPKHPIKHQQ	10	9
2a	RPKHPIKHQGL	11	10
3a	RPKHPIKHQGLP	12	11
A	RPKHPIKHQGLPQ	13	12
B	RPKHPIKHQGLPQB	14	13
C	RPKHPIKHQGLPQEV	15	14
D	RPKHPIKHQGLPQEVN	16	15
E	RPKHPIKHQGLPQEVLN	17	16
F	RPKHPIKHQGLPQEVNEN	18	17
G	RPKHPIKHQGLPQEVNEN	19	18

		78	
H	RPKHPIKHQGLPQEVLENENL	20	19
I	RPKHPIKHQGLPQEVLENENLL	21	20
J	RPKHPIKHQGLPQEVLENENLLR	22	21
K	RPKHPIKHQGLPQEVLENENLLRF	23	22
L	RPKHPIKHQGLPQEVLENENLLRFF	24	23
M	RPKHPIKHQGLPQEVLENENLLRFFV	25	24
N	RPKHPIKHQGLPQEVLENENLLRFFVA	26	25
β 193-208	YQEPVLGPFVRGPFPII	16	28
κ 106-127	MAIPPKKNQDKTEIPTINFIAS	22	30

***Juvenile (Type I, IDDM) diabetes in Non-Obese Diabetic (NOD) mice:***

***Peptides derived from natural casein:*** NOD mice are a commonly used model for research of autoimmune disease and human Juvenile Diabetes. Six week old female NOD mice received either one or two injections per week of 100 µg of peptides derived from natural casein, for a total of 5 or 10 treatments. Control mice received no treatment. The severity of disease was determined according to glucosuria, which was measured using Combi test sticks [Gross, D.J. *et al.* (1994), *Diabetology*, 37:1195]. Results were expressed as the percent of glucosuria-free mice in each sample over a 365-day period.

***Synthetic peptides derived from casein:*** In another experiment, 6 week old female NOD mice received one injection per week of 100 µg of Synthetic peptides derived from casein for a total of 5 treatments. Control mice received no treatment. Results were expressed as the number of healthy mice in the various treated groups.

***Intraperitoneal Glucose Tolerance Test (IPGTT):*** The glucose tolerance test is the definitive method for investigating glucose metabolism and diabetic tendencies in mammals. Twenty five (25) weeks after receiving Synthetic peptides derived from casein, response to a glucose load was assessed with an intraperitoneal glucose tolerance test. Glucose injection consisted of 1g/kg body weight. Glycemic values were determined from blood drawn prior to test (0 minutes) and 60 minutes after loading. Plasma glucose levels were

determined with a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA) and expressed as mmol/L. Normal values do not exceed 140 mmol/L.

***Stimulation of proliferation of Natural Killer (NK) cells:***

***From human Peripheral Blood Stem Cells (PBSC):*** PBSC of G-CSF treated subjects were separated on a FICOLL gradient, washed twice with RPMI-1640 medium containing 10% FCS and glutamine, and seeded into 1.5 ml wells with or without peptides derived from natural casein or synthetic peptides derived from casein, as indicated, (0-500 µg per ml). Following two days incubation the cells were assayed for Natural Killer activity by measuring radioactivity released from <sup>35</sup>S-labeled K562 target cells (NEG-709A, 185.00 MBq, 2.00 mCi EASYTAGth Methionine, L-[<sup>35</sup>S] 43.48 TBq per mmol, 1175.0 Ci per mmol, 0.488 ml, Boston USA). Two concentrations of effector cells ( $2.5 \times 10^5$  and  $5 \times 10^5$  cells per well) were incubated with  $5 \times 10^3$  target cells per well (effector:target cell ratios of 50:1 and 100:1, respectively) in U-bottomed 96 well tissue culture plates. The cells were incubated for 5 hours at 37 °C in 5 % CO<sub>2</sub>, 95 % air and precipitated by 5 minutes centrifugation at 1000 rpm. <sup>35</sup>S release was measured in 50 µl samples of the supernatant liquid.

***From murine Bone Marrow (BM) cells:*** Bone marrow was collected from 4 untreated BALB/c and C57Bl/6 mice. Bone marrow was harvested from the long bones of front and hind limbs of the mice by injection of medium using a 25 Gauge needle. Aspirated cells were washed with RPMI 1640, counted in a haemocytometer and vital-stained (20 µl of cells in 380 µl acetic acid/trypan blue), then seeded in culture bottles at  $2-5 \times 10^6$  cells per ml in RPMI-1640 containing 10 % Fetal Calf Serum, antibiotics and glutamine with or without 100 µg per ml peptides derived from natural casein. The cell cultures were incubated in 5 % CO<sub>2</sub>, 95 % air for 12-15 days at 37 °C, harvested by 10 minutes centrifugation at 1500 rpm, counted, and seeded in U-bottom wells with <sup>51</sup>Cr (Chromium-51, 740 MBq, 2.00 mCi activity) or <sup>35</sup>S (NEG-709A, 185.00 MBq, 2.00 mCi EASYTAGth Methionine, L-[<sup>35</sup>S] 43.48

80

TBq per mmol, 1175.0 Ci per mmol, 0.488 ml, Boston USA) labeled murine lymphoma (YAC) cells at either 25:1 or 50:1 effector:target cell ratio. NK activity is expressed as the percent radioactivity in the cell-free supernatants.

*Proliferation of human cells in culture:* Peripheral blood (PB) was collected from healthy or affected patients. Affected patients received no treatment other than G-CSF supplementation prior to plasmapheresis. Bone marrow (BM) cells were collected from consenting healthy patients or affected patients in remission following chemotherapy by aspiration. Umbilical cord blood was collected during normal births. Human cells of the various origins were separated on a FICOLL gradient, washed twice with RPMI-1640 medium, and seeded into 0.2 ml flat bottom tissue culture wells at the indicated concentrations with or without peptides derived from natural casein or with or without synthetic peptides derived from casein, as indicated. All treatments, including controls, were repeated in triplicate. Cell proliferation was measured by <sup>3</sup>HT incorporation: radioactive thymidine was added [thymidine (methyl-<sup>3</sup>H)] Specific activity 5 Ci per ml 37 MBq per ml, ICN Corp.] following incubation for the indicated number of days. Cells were then incubated 16-20 hours with the label, harvested and washed with medium. Incorporated radioactivity was measured in a  $\beta$  scintillation counter.

*Proliferation of K562 leukemia and colon cancer cell lines:* Colon and K562 are established lines of cancer cells grown in culture. Both cell lines were grown in culture bottles in 5 % CO<sub>2</sub>, 95 % air at 37 °C, harvested and washed with medium before seeding in tissue culture wells at 4 x 10<sup>5</sup> cells (K562) or 3 x 10<sup>3</sup> cells (Colon) per well. Peptides derived from natural casein were added to the wells, at the indicated concentrations, and after 9 (K562) or 3 (Colon) days of incubation labeled thymidine was added as described above. Harvesting and measurement of radioactive uptake was as described above.

*Fluorescent antibody detection of NK and T Cell proliferation in human Peripheral Blood Stem Cells (PBSC):*

Peripheral Blood Stem cells (PBSC) from human subjects receiving G-CSF treatment were collected by plasmapheresis, separated on a FICOLL gradient, washed twice with RPMI-1640 medium containing 10 % Fetal Calf Serum and incubated in culture bottles at 37 °C in 5 % CO<sub>2</sub>, 95 % air with or  
5 without peptides derived from natural casein at the indicated concentrations. Following 10, 14 or 28 days incubation with peptides derived from natural casein, the presence of T cells (CD<sub>3</sub> surface antigen) and NK cells (CD<sub>56</sub> surface antigen) was detected by direct immunofluorescence using anti-CD<sub>3</sub> fluorescent antibody (CD<sub>3</sub>/FITC clone UHCT<sub>1</sub>), anti-CD<sub>56</sub> fluorescent  
10 antibody (CD<sub>56</sub>/RPE clone MOC-1) (DAKO A/S, Denmark) and mouse IgG1/RPE and IgG1/FITC antibodies as a control. Detection of fluorescently tagged cells was performed using fluorescence activated cell sorting (FACS).



***Stimulation of hematopoiesis from Bone Marrow (BM) Cells in culture:***

***Proliferation of megakaryocytes in multipotential colonies (CFU-GEMM) from murine Bone Marrow cells:*** Primary bone marrow cells (1 x 10<sup>5</sup> per ml) from 8-12 week-old C3H/HeJ mice were grown in serum-free methyl cellulose-IMDM medium for 8-9 days at 5 % CO<sub>2</sub>, 95 % air, at 37 °C. The medium, appropriate for the growth of multipotential colonies (CFU-GEMM), contained 1 % BSA (Sigma), 10<sup>-4</sup> M thioglycerol (Sigma), 2.8 x 10<sup>-4</sup> M human transferrin (TF, Biological industries, Israel), 10 % WEHI-CM as a source of IL-3 and 2 units per ml erythropoietin (rhEPO, R & D Systems, Minneapolis). Colonies were scored after 8-9 days using an Olympus dark field microscope. They were picked with a micropipette, cytocentrifuged and stained with May-Grunwald-Giemsa for differential counts. At least 700 cells were counted for each preparation.

***Proliferation of Dendritic cells in CFU-GEMM:*** Multipotent (CFU-GEMM) colonies grown from primary bone marrow cells as described for the assay of megakaryocyte proliferation above were collected, stained and counted for dendritic cells. At least 700 cells were counted for each preparation.

**Proliferation of Plasma cells in CFU-GEMM:** Multipotent (CFU-GEMM) colonies grown from primary bone marrow cells as described for the assay of megakaryocyte proliferation above were collected, stained and counted for plasma cells. At least 700 cells were counted for each preparation.

5 **Proliferation of Macrophage cells in CFU-GEMM:** Multipotent (CFU-GEMM) colonies grown from primary bone marrow cells as described for the assay of megakaryocyte proliferation above were collected, stained and counted for macrophage cells. At least 700 cells were counted for each preparation.

10 **Proliferation of Red Blood Cells in CFU-GEMM:** Multipotent (CFU-GEMM) colonies grown from primary bone marrow cells as described for the assay of megakaryocyte proliferation above were collected, stained and counted for red blood cells. At least 700 cells were counted for each preparation.

15 **Proliferation of Polymorphonuclear Cells (PMN) in CFU-GEMM:** Multipotent (CFU-GEMM) colonies grown from primary bone marrow cells as described for the assay of megakaryocyte proliferation above were collected, stained and counted for polymorphonuclear cells. At least 700 cells were counted for each preparation.

20 **Proliferation of megakaryocyte- and erythroid forming cells from human bone marrow and cord blood cells:** A sample of bone marrow from an apparently healthy human being was processed by density gradient separation using Histopaque-107 (Sigma Diagnostics) to obtain a purified population of mononuclear cells (MNC). Colony assays were performed in a plating medium containing final concentrations of 0.92 % methyl cellulose (4000 centripase powder, Sigma Diagnostic), rehydrated in Iscoves modified Dulbecco's  
25 medium containing 36 mM sodium bicarbonate (Gibco), 30 % fetal bovine serum (FBS) (Hyclone), 0.292 mg/ml glutamine, 100 units per ml penicillin and 0.01 mg per ml streptomycin (Biological Industries, Beit Haemek). Cord blood from normal births was collected and prepared as mentioned above.

30 Colony assay medium containing  $10^5$  MNC per ml was plated in triplicate wells within a 24 well tissue culture plate (Greiner), 0.33 ml per well.

The cultures were incubated at 37 °C in 5 % CO<sub>2</sub>, 95 % air and 55 % relative humidity with or without peptides derived from natural casein or synthetic peptides derived from casein, at the indicated concentrations. Plates were scored after 14 days for colonies containing more than 50 cells.

5 Megakaryocytes were identified by indirect immunofluorescence using a highly specific rabbit antibody recognizing human platelet glycoproteins, and an FITC-conjugated goat anti-rabbit IgG. Added growth factors included 15 ng per ml leucomax (GM-CSF) (Sandoz Pharma), and 5 % vol. per vol. human phyto-hemagglutinin-m (Difco Lab)-induced conditioned medium (CM) to

10 induce development of granulocyte macrophage colonies (CFU-GM). Erythropoietin (EPO) 2 units/ml was used to induce formation of erythroid colonies (burst-forming unit-erythroid-BFU-E).

Alternatively, human bone marrow cells from consenting volunteer donors or patients undergoing autologous bone marrow transplantation were

15 precultured in medium containing 10-1000 µg per ml peptides derived from natural casein, grown in semi-solid agar, and scored for granulocyte-macrophage hematopoietic colonies (GM-CFU) at 7 or 14 days post treatment.

Megakaryocytopoiesis was measured in normal bone marrow cells from healthy consenting human donors by either scoring of the number of

20 megakaryocytes in samples of liquid culture (RPMI-1640 plus 10 % human AB serum, glutamine and antibiotics) with or without 100 µg per ml peptides derived from natural casein, or in a methylcellulose assay for assessing colony formation. 2 x 10<sup>5</sup> bone marrow cells were seeded in the presence of a standard growth factor combination with or without peptides derived from

25 natural casein. In the methylcellulose assay megakaryocytes were counted with an inverted microscope on days 12-14 after seeding.

*Clinical trials using peptides derived from natural casein:* In one series of trials, a single dose containing 50 mg peptides derived from natural casein was administered intra-muscular to human subjects in 3 depots, over a

period of 2 hours. Clinical parameters were monitored at the indicated intervals. In other trials, patients at various stages of treatment for and/or remission from cancer and metastatic disease received peptides derived from natural casein once or twice, and were monitored for changes in the cell count of peripheral blood.

***Inhibition of in vitro HIV infection of human lymphocyte cells:***

**Peptides:** Peptides (either peptides derived from natural casein or synthetic peptides derived from casein (2-26 amino acids in length, see table 3) supplied as lyophilized powder were resuspended in RPMI complete medium and added to cell cultures at a final concentrations of 50 to 1000 µg per ml.

**Cells:** Several types of freshly isolated human cells (primary cells) and cell lines are known to be susceptible to *in vitro* HIV-1 infection, although essentially any cell displaying even low surface levels of the CD<sub>4</sub> molecule can be considered a potential target for HIV-1 infection. Two commonly used human cell lines which are highly sensitive for HIV-1 infection were chosen, CEM and Sup-T1.

CEM is a human T4-lymphoblastoid cell line initially derived by G. E. Foley *et al.* [(1965), Cancer 18:522] from peripheral blood buffy coat of a 4-year old caucasian female with acute lymphoblastic leukemia. These cells were continuously maintained in suspension in medium, and have been used widely for analysis of infectivity, antiviral agents and neutralizing antibodies.

Sup-T1 is a human T-lymphoblastoid cell line isolated from a pleural effusion of an 8-year old male with Non-Hodgkin's T-cell lymphoma [Smith, S. D. *et al.* [(1984) Cancer Research 44:5657]. This cell expresses high levels of surface CD<sub>4</sub> and is useful in studies of cell fusion, cytopathic effect and infectivity of HIV-1. Sup-T1 cells are grown in suspension in enriched medium.

**Medium:** Cells were grown in RPMI-1640 complete medium enriched with 10 % Fetal bovine serum, 2 mM glutamine and 2 mM penicillin-streptomycin (GIBCO).

**Virus:** The HIV virus strain employed was HIV-1IIB, originally designated HTLV-III<sub>B</sub>. Concentrated culture fluids of peripheral blood from several patients with AIDS or related diseases were used to establish a permanent productive infection in H-9 cells. This subtype B virus has high capacity to replicate in human T-cell lines. Viral titer was 5.38 ng per ml in stock solution.

**FITC-labeled peptides:** FITC F-1300 (Fluorescein isothiocyanate, isomer I, Sigma (F250-2) St. Louis, MI, USA) having excitation/emission maxima of about 494/520 nm, respectively, was employed. The amine-reactive fluorescein derivative is probably the most common fluorescent derivatization reagent for covalently labeling proteins. FITC-conjugated peptides derived from natural casein were prepared by covalent binding of FITC to the amine groups of lysine.

**HIV-1 P<sup>24</sup> antigen capture assay:** An HIV-1 P<sup>24</sup> Antigen capture assay kit employed was designed to quantitate the HIV-1 P<sup>24</sup> core antigen, which is proportionally related to the degree of viral production in cells. This kit was purchased from the AIDS Vaccine program of the SAIC-NCI-Frederick Cancer Research Institute, P.O. Box B, Frederick, M.D 21702, USA and included 96 well plates coated with monoclonal antibody to HIV-1 P<sup>24</sup>, primary antibody-rabbit anti-HIV P<sup>24</sup> serum, secondary antibody-Goat anti-rabbit-IgG (H+L) peroxidase conjugated antibody, TMB peroxidase substrate system and lysed HIV-1 P<sup>24</sup> standard. The HIV-1 P<sup>24</sup> antigen capture assay was analyzed by Organon-Technica ELISA reader at 450 nm with a reference at 650 nm.

**HIV-1 P<sup>24</sup> antigen capture ELISA:** HIV infection was measured with an indirect enzyme immunoassay which detects HIV-1 P<sup>24</sup> core antigens in tissue culture media. Tissue culture supernatant was reacted with primary rabbit anti-HIV-1 P<sup>24</sup> antigen and visualized by peroxidase conjugated goat anti

rabbit IgG. The reaction was terminated by adding 4N H<sub>2</sub>SO<sub>4</sub>, wherein the intensity of the color developed is proportional to the amount of HIV-1 antigen present in the tissue culture supernatant.

**Biological hazard level 3 (BL-3) laboratory:** All virus production isolation and infection, tissue culture of HIV-1 infected cells, P<sup>24</sup> antigen containing supernatant harvesting and P<sup>24</sup> antigen capture ELISA, were performed in BL-3 facility and were in accordance with the bio safety practices set by the NIH and CDC (USA).

**Flow cytometry:** A FACSort cell sorter (Becton & Dickinson, San Jose, CA, USA) was used to (i) determine the percentage of CD4 positive CEM and sup-T1 cells batches before infection with HIV-1 in order to assure the same degree of infection in each experiment; and (ii) detect T cells that harbor FITC conjugated peptides derived from natural casein in their cytoplasm and nuclei.

**CO<sub>2</sub> incubator:** For viral culture production cells with HIV-1, cells and virus pretreated with peptides derived from natural casein and cells which were further incubated with HIV-1, were all kept in humidified CO<sub>2</sub> incubator for the duration of the experiment.

**HIV infection of human cultured CD4 cells:** For longer incubations, the cells (CEM, Sup-T1) were preincubated with several increasing concentrations of peptides derived from natural casein (50-1000 µg per ml) or synthetic peptides derived from casein (10-500 µg per ml) for 24 (for synthetic and natural peptides) and 48 (only for natural peptides) hours and HIV-1IIIB (45 pg per ml final concentration) was added to each well thereafter. For the shorter incubations (3 hours), HIV-1IIIB was preincubated with the peptides for 3 hours and then added to cells (5000 cells/ well) in tissue culture plates. Controls were IF (Infected, cells cultured with HIV-1 and without peptides), UIF (Uninfected, cells cultured without HIV-1 and without peptides) and UIF + Ch (Uninfected + peptides derived from natural casein, cells cultured in the presence of peptides derived from natural casein {50-1000 µg per ml}) to test

the effect of peptides derived from natural casein and synthetic peptides derived from casein on cell viability and growth. Cells were counted for viability and proliferation rate on day 7, 10 and day 14 post infection (the day of P<sup>24</sup> antigen culture supernatant harvest). Cells and tissue culture supernatants (media) were harvested and lysed immediately in 1/10 volume of 10 % Triton X-100. These samples were further incubated at 37 °C for 1 hour and kept at -80 °C until tested for P<sup>24</sup> antigen.

**Confocal microscopy:** A Zeiss LSM 410 confocal laser scanning system attached to TW Zeiss Axiovert 135M inverted microscope, employing the laser scanning confocal microscopy technique, was used to detect penetration of FITC conjugated peptides into cells. T cells were incubated with FITC conjugated peptides derived from natural casein in a 5 % CO<sub>2</sub>, 95 % air, 37 °C incubator, after which the cells were washed 3 times with phosphate buffer saline (PBS) to remove unbound FITC-peptides. Cells were fixed with 3.8 % formalin for 10 minutes, washed twice with PBS and resuspended in 50-100 µl PBS before viewing the cells under the microscope. Selected images of cells from different time points of incubation (15 minutes, 30 minutes, 1 hour, 1.5 hour and 3 hours) displaying various amounts of FITC-peptides derived from natural casein in their cytoplasm and nuclei were stored on 3.5" Zip drive (230 MB) and processed for pictures using Photoshop software.

**[<sup>3</sup>H]-thymidine incorporation test:** In order to test the effect of peptides derived from natural casein on T cell proliferation, several concentrations of peptides derived from natural casein (10 mg/ml stock in RPMI) were added to Sup-T1 cell cultures in 96 flat bottom microwell plate (5000 cells/well), as described for HIV-1 infection in Sup-T1 cells. Cells were counted and their viability was determined by trypan blue dye exclusion. They were pulsed with [<sup>3</sup>H]-thymidine at each time point (3, 7, 10 and 14 days) for 18 hours (over night) and harvested on glass fiber filters for radioactivity reading

(Incorporation of [ $^3$ H]-thymidine into cellular DNA is proportional to degree of cell proliferation).

***Toxicity of peptides derived from natural casein in normal, myeloablated and transplant recipient mice and guinea pigs:*** Intramuscular, or intravenous injections of up to 5,000 mg peptides derived from natural casein per kg animal were administered in a single dose, or in three doses to normal animals. A variety of strains were employed, including BALB/c, C3H/HeJ and Non-Obese Diabetic (NOD) mice. The mice were either monitored for 10 months before sacrifice and post-mortem examination (toxicity assay) or observed for 200 days (survival rate). Guinea pigs received a single intramuscular injection of 20 mg peptides derived from natural casein per animal. Fifteen days later they were sacrificed and examined for pathology.

***Leukocyte and platelet reconstitution in bone marrow transplant recipient mice:*** BALB/c mice were sub-lethally irradiated at a source to skin distance of 70 cm, dosage of 50 cGy per minute, for a total of 600 cGy. The irradiated mice were reconstituted with syngeneic bone marrow as described above and injected intravenously 24 hours later with 1 mg per animal peptides derived from natural casein, synthetic peptides derived from casein (13-26 amino acids, see Table 3 above), or human serum albumin (controls), following a double-blinded protocol. Leukocyte reconstitution was determined according to cell count in peripheral blood collected at indicated intervals from 6 to 12 days post treatment. Platelet reconstitution was determined by cell count in blood collected from the retro orbital plexus, into EDTA-containing vials, at indicated intervals from day 6 to day 15 post treatment.

In an additional series of experiments, CBA mice were lethally irradiated (900 cGy), reconstituted with BM cells and treated with peptides derived from natural casein or human serum albumin as described above. Platelet reconstitution was assayed as mentioned above.

In a third series of experiments, the mice were irradiated (800 cGy), reconstituted and injected intraperitoneally with 1.0 mg synthetic peptides



derived from casein (peptides 3a and 4P, representing the first 6 and 12 amino acids of the N terminus of  $\alpha$ S1 casein, respectively - see Table 3 above) daily, on days 4, 5, 6 and 7 post-transplantation. Platelet reconstitution was assayed at 10 and 12 days post-transplantation.

5 In a fourth series of experiments, F1 mice were irradiated (750 cGy), reconstituted with syngeneic bone marrow, and injected intravenously 24 hours later with 1mg per mouse of synthetic peptides derived from casein representing amino acids 193-208 of  $\beta$ -casein and amino acids 1-22 of the N terminus of  $\alpha$ S1 casein. In addition, 2 (two) groups of mice were treated each  
10 with a natural fraction of  $\alpha$ S1 casein position 1-23, and a fraction of peptides derived from natural  $\kappa$ -casein, representing amino acid coordinates 106-169 of  $\kappa$ -casein (SEQ ID No. 30). WBC counts were conducted on days 5, 7, 10 and 12 post-transplantation.

15 ***Reconstitution of bone marrow transplant recipient mice and enhancement of bone marrow cell proliferation in donor mice:***

C57Bl/6 mice were lethally irradiated at a source to skin distance of 70 cm, dosage of 50 cGy per minute, for a total of 900 cGy. The irradiated mice were reconstituted with syngeneic bone marrow cells from mice which were either treated a day prior to bone marrow collection with 1 mg per animal  
20 peptides derived from natural casein or with saline (controls), following a double-blinded protocol. In one experiment mice survival was monitored for 18 days. In another experiment mice were sacrificed after 8 days and spleen colonization monitored.

25 ***Synthetic peptides derived from casein significantly reduce Cholesterol levels:***

The ability of synthetic casein derived peptides to reduce cholesterol levels in 7-week old female C57Bl/6j mice was assessed after feeding an atherogenic diet. The mice were divided into groups of 8. One control group was fed a normal diet. A second control group was fed the modified Thomas  
30 Hartroft diet containing cholate (#TD 88051: Teklad, Madison, WI) [Gerber, D.

W. *et al.*, Journal of Lipid Research. 42, 2001]. The remaining experimental groups were all fed the modified Thomas Hartroft diet. After one week on the diet, serum cholesterol values increased significantly and the synthetic peptides derived from casein were injected intraperitoneally, 1 mg per mouse, followed  
5 by a second injection of 0.1 mg one week later.

Cholestrol blood levels were determined according to Roche Cholesterol Assay based on Roeschlou & Allin enzymatic method (Roche, Inc., Germany).

### EXPERIMENTAL RESULTS

10 **Peptides derived from natural casein:** Originating from the observation that curdled milk occasionally failed to support bacterial growth, a casein fragment possessing bacteriocidal properties was isolated from milk proteins (United States Patent No. 3,764,670 to Katzirkatchalsky, *et al.*). Crude peptides derived by proteolysis of natural casein were prepared by acid precipitation of  
15 the soluble fraction of the casein proteolytic digest, dialysis and lyophilization. When tested for biological activity after extended storage, it was noted that this crude preparation, when lyophilized and stored at 4 °C, remained active (*in vitro* and *in vivo*) for at least 24 months.

**Low Temperature-Processed Peptides from natural casein:**  
20 Preparation of the casein hydrolysate according to traditional methods, such as that described by Hill *et al.*, requires high temperature (>75 °C) inactivation of the proteolytic enzymes, a time consuming process resulting in irreversible denaturation of the large amounts of proteolytic enzymes required for the production of Peptides from natural casein, and potential unknown effects on  
25 the hydrolysate itself. While reducing the present invention to practice, it was surprisingly discovered that the proteolytic process producing peptides from natural casein can be terminated more efficiently, by a novel, simplified method comprising cooling, alkaline treatment, and subsequent acidification.

In a representative preparation, and to compare Low-Temperature  
30 Processing with the conventional heat treatment, a 1.7% casein solution

prepared as described hereinabove was subjected to proteolytic digestion with a proteolytic enzyme (for example, chymosin (known also as renin) either as crystalline renin or commercial chymosin of non-animal source. Other proteolytic enzymes, as pepsin, can also be used).

5        20 ng of the enzyme was added per each ml of the 1.7% casein solution. Proteolytic digestion of the casein was completed after 14.5 hours at 30°C.

At the completion of the reaction, the reaction mixture was cooled immediately to below 10°C, made 2% with cold TCA (Tri-chloro acetic acid), and maintained below 10°C. Following removal and filtration of the resulting  
10 supernatant, which still contained most of the peptides derived from natural casein, the supernatant was made 10- 12.5% in cold TCA, and centrifuged at 1370xg at below 10°C.

The resulting precipitate comprising peptides derived from natural casein was removed and dissolved in H<sub>2</sub>O and made strongly basic (pH 9-13)  
15 with an alkaline solution. The solution was kept at this basic pH between 15 minutes to 1 hour, and then acidified with HCl, to a final pH of between pH 7-9. Further purification of peptides was performed by gel filtration or diafiltration, as described hereinabove.

Surprisingly, it was observed that maintaining the solution at an alkaline  
20 pH (between pH 9-13) for sufficient time (from 15 minutes to 1 hour), terminated enzymatic activity completely, and caused an irreversible denaturation thereof.

In order to identify the active peptides contained in the peptides derived from natural casein the lyophilized preparation was fractionated using high  
25 performance liquid chromatography (HPLC), as described hereinabove. All of the lyophilized samples analyzed demonstrated similar retention time profiles, with contents as described above.

Thus, major components of the crude peptides derived from natural casein preparation are the N-terminal fragment of  $\alpha$ S1 casein, a peptide  
30 representing a fragment of  $\beta$  casein (SEQ ID No. 27), and a peptide

representing a fragment of  $\kappa$  casein (SEQ ID No. 30). Minor components identified are a fragment of the N-terminal portion of  $\alpha$ S1 casein, a peptide representing a further, distinct fragment of  $\alpha$ S1 casein (SEQ ID No. 31), a peptide representing a fragment of  $\alpha$ S2 casein (SEQ ID No. 32), and a peptide  
5 representing a further, distinct fragment of  $\alpha$ S2 casein (SEQ ID No. 33).

***Peptides derived from natural casein are non-toxic in rodents and humans:*** Extensive investigation of the short and long term effects of high doses of peptides derived from natural casein on mice, rats, guinea pigs and human volunteers confirmed the absence of toxicity, teratogenicity or adverse  
10 side effects of the preparation. In one series of tests, single doses representing 7,000 times the estimated effective dose of peptides derived from natural casein were administered intra muscularly to mice. Standard post-mortem pathology examination of the mice at 14 days post treatment revealed no toxic effects on internal organs or other abnormalities. Similar toxicity tests in guinea pigs  
15 revealed no abnormalities two weeks after single 20 mg intra-muscular doses of peptides derived from natural casein. In another series of experiments, high doses of peptides derived from natural casein administered to healthy mice had no effect on several hematological parameters measured two weeks later, including white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB),  
20 electrolytes, glucose and others. A third series of experiments tested repeated high doses of 100 mg per kg body weight in mice and rats for two weeks, revealing no allergic, delayed cutaneous or anaphylactic responses and no pathological effects upon post-mortem examination. When peptides derived from natural casein were tested for their effect on the long-term survival of  
25 irradiated, bone marrow reconstituted BALB/c and C3H/HeJ mice, survival of the treated mice (18 of 27 BALB/c and C3H/HeJ; 66 %) clearly exceeded the survival rates of the albumin-treated controls (4 of 26 BALB/c and C3H/HeJ; 15 %). Standard teratogenicity tests [for details see, for example, Drug Safety in Pregnancy, Folb and Dakes, p. 336, Elsevier, Amsterdam, NewYork, Oxford

(1990)] in mice treated with peptides derived from natural casein revealed no effect of the peptides on any developmental parameters.

Similar to its lack of toxicity or side effects when tested in rodents, peptides derived from natural casein were safe when administered to humans as well. Comparison of blood and urine samples from seven healthy human volunteers before, during and 7 days after intramuscular injection of peptides derived from natural casein revealed no changes in any of the clinical parameters. No other negative effects were observed.

Thus, high dose and extended treatment of rodents with peptides derived from natural casein revealed no apparent toxic, pathological, hypersensitivity, teratogenic, serological or any other negative effects. Moreover, peptides derived from natural casein administration to irradiated mice, at risk for short- and long-term complications, conferred a significant survival advantage over 200-300 days. These, and the absence of any undesirable effects in healthy human volunteers receiving peptides derived from natural casein via injections clearly demonstrate the peptide's safety in parenteral administration.

***Reconstitution of bone marrow in transplant recipient mice:*** When C57Bl/6 mice were lethally irradiated and reconstituted with syngeneic bone marrow from mice that were either treated a day prior to bone marrow collection with 1 mg per animal peptides derived from natural casein or not so treated, survival of irradiated mice that received bone marrow from treated mice far exceeded that of irradiated mice that received bone marrow from non treated mice (survival of irradiated mice that received bone marrow from treated mice was 15 out of 18, 10 days post irradiation; whereas survival of irradiated mice that received bone marrow cells from saline-treated control mice was 4 out of 17, 10 days post irradiation). Spleens derived from irradiated mice that received bone marrow from treated mice included about twice to three times as many colonies per spleen, as compared to spleens of irradiated mice that received bone marrow cells from saline-treated control mice (1-5 colonies as compared to 0-3 colonies).

***Peptides derived from natural casein stimulate the proliferation of lymphocytes:*** Natural killer (NK) and cytotoxic T cells are crucial to the immune system's ability to protect against invasion by both infectious pathogens and cancer cells, by both active cytotoxicity and the secretion of immunoregulatory lymphokines. Immune compromise, such as in AIDS or following chemotherapy, results in abnormal, weakened T or NK cell activity. When normal murine bone marrow cells from BALB/c and C57Bl/6 mice were cultured in the presence of 100 µg per ml peptides derived from natural casein, a clear increase in NK activity was observed in both effector:target cell ratio groups. Moreover, comparison between the two groups revealed a clear dose response relationship. At the 25:1 effector:target cell ratio the average NK activity was elevated from 13.93 % to 30.77 % and at the 50:1 effector:target cell ratio the average NK activity was elevated from 13.68 % to 44.05 % (Figure 1). Similar experiments using human Peripheral Blood Stem Cells from Granulocyte Colony Stimulating Factor-treated donors demonstrated an even more significant, concentration- dependent stimulation of target cell lysis by peptides derived from natural casein.

In the first set of experiments (Figure 2a), NK activity was measured in blood samples taken from one patient and incubated at two effector:target cell ratios with increasing peptides derived from natural casein concentration. Only 4 % <sup>35</sup>S release was measured in the control, untreated PBSC culture. Almost the same percent radioactivity (4%) was found at the lowest peptide concentration (5µg per ml). However, at higher peptide concentrations, in the range of 10µg per ml up to 100µg per ml, a release of 10.8-14.9 % <sup>35</sup>S was measured for effector:target cell ratios of 100:1 and 8.3-14.5 % <sup>35</sup>S for effector target cell ratios of 50:1 (Figure 2a).

When PBS cells from normal (patient 1) and affected (patients 2-6) human donors were incubated with increasing concentrations of the peptides derived from natural casein, a significant enhancement of affected patients' NK

cell activity could be measured. Thus, while the peptides derived from natural casein had a minimal effect on the normal patient's NK activity (increased from 13- 15 %  $^{35}\text{S}$  release, patient 1), PBS cells from both breast cancer and Non-Hodgkins Lymphoma patients (patients 3 and 4, for example) exhibited dramatic, dose-dependent increases in NK activity (3.5 to 10.8 %  $^{35}\text{S}$ ; 12.2 to 19.1 %  $^{35}\text{S}$ , respectively) (Figure. 2b).

***Peptides derived from natural casein stimulate the proliferation of CD56 surface antigen positive (NK) cells:*** In another series of experiments Peripheral Blood Stem Cells (PBSC) from 5 human donors receiving G-CSF treatment were incubated with peptides derived from natural casein for 10, 14, or 28 days, then assayed for presence of the CD<sub>56</sub> antigen. A sometimes dramatic increase in CD<sub>56</sub> antigen detection was observed in the peptide-treated cells from all the donors but one (patient 1). A representative response is depicted in Figure 3a: Following 10 days of incubation with or without peptides derived from natural casein, the presence of CD<sub>56</sub> surface antigen-positive (NK) cells was detected by direct immunofluorescent staining. Overall, incubation with peptides derived from natural casein increased the mean percentage of the cells positively stained for CD<sub>56</sub> from 0.64 % in the control group to 2.0 % following treatment (Figure 3a).

***Peptides derived from natural casein stimulate the proliferation of CD3 surface antigen-positive (T) cells:*** The effect of peptides derived from natural casein on the proliferation of CD<sub>3</sub> surface antigen-positive (T) cells in PBS cells from 5 subjects was assayed by direct immunofluorescence. In all but one patient (patient 4), 14 days incubation with peptides derived from natural casein significantly increased T-cell proliferation, up to more than 5 fold in some. Taken together, the mean percentage of the cells positively stained for CD<sub>3</sub> increased from 19.45 % in the control group to 35.54 % in the treated group (Figure 3b).

***Peptides derived from natural casein stimulate the proliferation of - CD56 and CD3 (NK/ T-cells) positive cells:*** In an additional experiment

PBSCs from 7 patients were incubated with peptides derived from natural casein for 28 days, and the effect on proliferation of NK/T cells (CD<sub>56</sub> and CD<sub>3</sub> surface antigen-positive) was detected by direct immunofluorescence. Incubation with peptides derived from natural casein stimulated proliferation of T-cell greater than 5 fold in some cases (patient 6), while the mean percentage of the CD<sub>3</sub>- positive (T-) cells increased from 2.08 % in the control group to 6.49 % in the treated group. The number of both CD<sub>56</sub> and CD<sub>3</sub> surface antigen-positive (NK/T) cells was increased from 1.1 % in the control to 4.3 % in the treated group (Figure 3c). Thus, peptides derived from natural casein stimulate the proliferation of both T-lymphocytes and Natural Killer cells from normal murine and human blood cell progenitors. Significantly, the greatest immunestimulatory effect of the peptides derived from natural casein was noted in human donors having initially low T- and NK cell levels (Figures. 3a-c).

***Synthetic peptides derived from casein stimulate human lymphocyte proliferation in vitro:*** When synthetic peptides derived from casein representing the first 3 to 26 residues of  $\alpha$ S1 casein were incubated with human PBSCs from healthy and cancer patients (see below), a significant increase in NK cell activity was observed. Target cell lysis was greatest (from 3 to greater than 5 fold that of controls) in Non-Hodgkin's Lymphoma and Breast Cancer patient's PBSC cultures after two days incubation with as little as 10  $\mu$ g per ml of peptides containing the first 9 or more residues of  $\alpha$ S1 casein (Figure 4). Under identical conditions, none of the peptides tested had a significant effect on NK activity in PBSC cultures from healthy human donors. Thus, even low concentrations of peptides containing the first 10 residues of the N-terminal sequence of  $\alpha$ S1 casein are capable of selectively stimulating *in vitro* lymphocyte proliferation in cells from cancer patients.

Similar stimulation of NK cell activity was observed when PBS cells from human donors with hematopoietic disease were incubated with Synthetic peptides derived from casein representing the first 3 amino acid residues of  $\alpha$ S1



casein. Incubation of the PBS cells with the peptides increased target cell lysis from 2- to greater than 8- fold that of the untreated controls. Of the 5 patients tested, three (3) responded to 25 µg/ml peptide concentration, one (1) responded to 100 µg/ml peptide concentration and one (1) to 250 µg/ml. Three out of the five (5) patients responded at 25 µg/ml.. No significant effect on NK activity in PBSC cultures from healthy human donors treated with the synthetic peptide representing the first 3 amino acids of αS1 casein, was observed, confirming the selective nature of the human lymphocyte-stimulating properties of casein-derived peptides.

10        ***Stimulation of hematopoiesis in human blood cell progenitors:***

Blood cell progenitors differentiate into a variety of blood cells: macrophages, monocytes, granulocytes, lymphocytes, erythrocytes and megakaryocytes. Progenitor cells are abundant in bone marrow, but are also found in peripheral blood after Granulocyte Colony Stimulating Factor treatment (PBSCs), and fresh Cord Blood. When increasing concentrations (50-600 µg per ml) of peptides derived from natural casein were added to cultures of human Bone Marrow, PBSC and Cord Blood, an increase in cell proliferation, as measured by [<sup>3</sup>H]-thymidine incorporation was noted (Figures 5a-5c). Human PBSC proliferation was most greatly effected by 300 µg per ml (Figure 5a) after 15 days in culture. An even greater effect was noted for Cord Blood cells in culture (3 to 4 fold increase in [<sup>3</sup>H]-thymidine incorporation) after 14 days incubation (but not after 7 days) with peptides derived from natural casein (600 µg per ml, Figure 5c). Cultured human bone marrow cells from three out of four donors also reacted strongly (3 to 5 fold increase in incorporation) to peptides derived from natural casein (300 µg per ml) after 21 days incubation (Figure 5b). Thus, peptides derived from natural casein stimulate proliferation of human blood cell progenitors from bone marrow as well as other sources. Interestingly, incubation of cultured human K562 (Chronic Myeloid Leukemia) and Colon (Colon cancer) cell lines with high

concentrations (up to 500 µg per ml) of peptides derived from natural casein under similar conditions had no effect on [<sup>3</sup>H]-thymidine incorporation. Thus, peptides derived from natural casein stimulate proliferation of human blood cell progenitors but not growth of cancerous cells in vitro.

5     ***Stimulation of megakaryocytopoiesis by peptides derived from casein:***

***Peptides derived from natural casein stimulate megakaryocyte progenitor proliferation in cultured murine bone marrow cells:***

      Multinucleated megakaryocytes develop in the bone marrow from primitive stem cells, mature to giant cells and give rise to thousands of thrombocytes per  
10    megakaryocyte. Thrombocytes are crucial for clot formation and thrombocytopenia is a major concern in myeloablative conditions (following chemotherapy or radiotherapy).

      Primary bone marrow cell cultures can be induced to form CFU-GM (Granulocyte and Monocyte) colonies, and CFU-GEMM (Granulocyte,  
15    Erythroid, Macrophage and Megakaryocyte) colonies, containing additional blood cell types. Colony counts reflect expansion of specific progenitors, cell numbers reflect proliferation rates and differential cell counts reflect which specific cell lineages have developed [Patenkin, D. *et al.* (1990), Mol. Cel. Biol. 10, 6046-50]. In cultured murine bone marrow cells incubated with  
20    erythropoietin and IL-3, addition of 25 µg per ml peptides derived from natural casein for 8 days increased the number of CFU-GEMM two and one half fold over controls, stimulating a three fold increase in relative cell numbers per colony in the CFU-GEMM. In a similar series of experiments, addition of  
25    peptides derived from natural casein to bone marrow cells incubated with erythropoietin and conditioned medium (see Materials and Experimental Methods) stimulated a concentration-dependent increase in the percentage of early and late megakaryocytes (15 % megakaryocytes without peptides, to 50 % with 500 µg per ml peptides derived from natural casein). Thus, 8 days treatment with peptides derived from natural casein stimulated a significant

increase in megakaryocyte formation and development in primary murine bone marrow cultures.

In a similar series of experiments, synthetic  $\alpha$ S1-,  $\alpha$ S2-,  $\beta$ - or  $\kappa$ -casein, alone or in combination, stimulated proliferation of GEMM colonies in cultured primary murine bone marrow cells. The number of GEMM colonies scored in murine bone marrow cells prepared as above and exposed to 25  $\mu$ g per ml synthetic peptides derived from  $\beta$ - (SEQ ID NO: 28) or  $\kappa$ - (SEQ ID NO: 30) casein, was greatly enhanced (>100%) at 8 days incubation compared with untreated (0  $\mu$ g per ml) control colonies (Fig. 22). Surprisingly, the two peptides in combination exerted an even greater effect on GEMM colony formation. Exposure of the murine primary bone marrow cells to a combination of optimal concentrations of peptides derived from  $\beta$ - (SEQ ID NO: 28) and  $\kappa$ - (SEQ ID NO: 30) casein ( $\beta$ + $\kappa$ ) unexpectedly resulted in a strongly enhanced effect on GEMM proliferation (>350%, Fig. 22). Thus, peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein-casein are more effective in stimulating GEMM proliferation in combination than each alone.

***Synthetic peptides derived from casein stimulate megakaryocyte progenitor proliferation in cultured murine bone marrow cells:***

Similar to the above and under similar experimental conditions, synthetic peptides derived from casein representing the first 5 to 24 amino acids of  $\alpha$ S1casein increase the percentage of early and late megakaryocytes from 15 % without the synthetic peptide to more than 40 % with 25  $\mu$ g per ml of synthetic peptides (Figure 7). Thus, 8 days treatment with synthetic casein derived peptides representing the first 5, 6, 11, 12, 17, 18, 19, 20, 21 and 24 amino acids stimulated a significant increase in megakaryocyte formation and development in primary murine bone marrow culture. Somewhat milder, yet appreciable, stimulation was observed with the other synthetic peptides derived from  $\alpha$ S1casein.

In a similar experimental regimen, synthetic peptides representing amino acids 193-208 of  $\beta$ -casein (SEQ ID NO. 28), amino acids 106- 127 of  $\kappa$ -casein (SEQ ID NO. 30), and amino acids 1-22 of  $\alpha$ S1-casein (SEQ ID NO. 21) all stimulated an increase in early, late and total megakaryocyte formation and development in primary murine bone marrow cultures. An increase in total megakaryocyte proliferation of 21% , 32% and 57% over controls was observed in cells supplemented with synthetic  $\kappa$ -casein (SEQ ID NO. 30),  $\beta$ -casein (SEQ ID NO. 28), and  $\alpha$ S1-casein (SEQ ID NO. 21), respectively (Figure 21).

***Peptides derived from natural casein stimulate Megakaryocytopoiesis in cultured human bone marrow cells:*** When 100  $\mu$ g per ml peptides derived from natural casein were added under similar conditions to human bone marrow cell cultures from healthy donors, CFU-GM colony formation was increased with or without additional stimulating factors (GM-CSF, CM). Peptides derived from natural casein also stimulated erythroid cell forming colonies in the presence of erythropoietin. Treatment of the human bone marrow cells with thrombopoietin (TPO) stimulates megakaryocyte (MK) colony formation. Addition of 300  $\mu$ g per ml peptides derived from natural casein to TPO-treated cells stimulates a more than twofold increase (16 colonies per  $2 \times 10^5$  cells without peptides, 35 colonies per  $2 \times 10^5$  with peptides derived from natural casein) in MK colony proliferation.

In the presence of additional hematopoietic factors, such as erythropoietin, human IL-3, hSCF and AB serum, 14 days incubation with peptides derived from natural casein stimulated a nearly three fold increase in CFU-GEMM colonies from human bone marrow cells (158 colonies with 500  $\mu$ g per ml peptides derived from natural casein, 68 colonies with the factors alone), but had a smaller (one and one half fold) effect on cultured cord blood CFU-GEMM formation. The relative cell number counts in the cultured human bone marrow and cord blood colonies reflect megakaryocyte cell proliferation in response to addition of 25  $\mu$ g per ml peptides derived from natural casein

(see Table shown in Figure 6). Thus, incubation of cultured human primary bone marrow and cord blood cells with peptides derived from natural casein stimulates the development and proliferation of both committed megakaryocyte and erythroid cell colonies. Significantly, the synergy observed between TPO and peptides derived from natural casein in stimulating megakaryocytopoiesis indicates a probable role for this potent hematopoietic growth factor in the mechanism of peptides derived from casein's stimulatory properties, and further suggests the likelihood of similar augmentation of a wide range of TPO-mediated effects by peptides derived from natural casein.

***Peptides derived from natural casein and synthetic peptides derived from natural casein potentiate the effect of Erythropoietin (EPO) in cultured human bone marrow cells:*** The effect of natural and synthetic peptides derived from casein on erythroid cell proliferation in cultured human bone marrow cells was assessed under the same conditions outlined hereinabove for megakaryocytopoiesis. When added in the presence of EPO, 50 -300 µg/ml peptides derived from natural casein, or 100 µg/ml Synthetic peptides derived from casein (F, Table 3, SEQ ID NO:18) stimulated a one and one-half (synthetic peptide) to four-fold proliferation of erythroid cell precursors (appearance of BFU-E colonies) compared to the bone marrow cells treated with EPO alone. Thus, peptides derived from natural casein and synthetic derivatives thereof act to potentiate the erythropoietic-stimulating effects of EPO, and as such can be used to augment of a wide range of clinically important EPO-mediated effects.

***Synthetic peptides derived from casein stimulate Dendritic cells proliferation in murine CFU-GEMM:*** The effect of Synthetic peptides derived from casein on dendritic cell proliferation in murine primary bone marrow cells was assessed under the same conditions outlined for the stimulation of megakaryocytes. Synthetic peptides derived from casein representing the first: 2, 3, 5, 6, 7, 9, 11, 12, 16, 23, 24 and 26 amino acids of α S1 casein stimulated the proliferation of dendritic cells, from 2.2 % and up to

23 % of total cells compared with 0.1 – 0.2 % dendritic cells in the cell samples incubated without Synthetic peptides derived from casein (Figure 7).

***Synthetic peptides derived from casein stimulate Plasma cell proliferation in murine CFU-GEMM:*** The effect of Synthetic peptides  
5 derived from casein on plasma cell proliferation in murine primary bone marrow cells was demonstrated under the same conditions outlined for the stimulation of megakaryocytes. Synthetic peptides derived from casein representing the first: 2, 3, 5, 7, 11, 16, 17, 18, 19, 20, 21, 22, 23 and 24 and 26  
10 amino acids of  $\alpha$ S1 casein, significantly stimulated the proliferation of plasma cells, from 1.5 % and up 12.3 % of total cell count, compared with 0.3 % of total without Synthetic peptides derived from casein (Figure 7).

***Synthetic peptides derived from casein stimulate Macrophage proliferation in CFU-GEMM:*** The effect of Synthetic peptides derived from  
15 casein on macrophage proliferation in murine primary bone marrow cells was demonstrated under the same conditions outlined for the stimulation of megakaryocytes. Incubation of cells with synthetic peptides derived from casein representing the first: 7, 9, 16, and 23 amino acids of  $\alpha$ S1casein significantly stimulated the proliferation of macrophages, from approximately  
17 % of total cell count in controls, to nearly 30 % of total in cells incubated  
20 with Synthetic peptides derived from casein (Figure 7).

***Synthetic peptides derived from casein stimulate Red Blood Cells proliferation in CFU-GEMM:*** The effect of Synthetic peptides derived from  
casein on red blood cell proliferation in murine primary bone marrow cells was demonstrated under the same conditions outlined for the stimulation of  
25 megakaryocytes. Incubation of cells with Synthetic peptides derived from casein representing the first 4 amino acids from the N terminus of  $\alpha$ S1casein (SEQ ID NO.3) significantly stimulated the proliferation of red blood cells, from 53 % of total cell count in controls, to 71 % of total in cells incubated with the synthetic peptide derived from casein (Figure 7).

***Synthetic peptides derived from casein stimulate Polymorphonuclear (PMN) cell proliferation in CFU-GEMM:*** The effect of Synthetic peptides derived from casein on the proliferation of polymorphonuclear (PMN) cells in murine primary bone marrow cells was demonstrated under the same conditions outlined for the stimulation of megakaryocytes. Incubation of cells with Synthetic peptides derived from casein representing the first: 3, 6, 7, 9, 16 and more, up to and including 26 amino acids of  $\alpha$ S1 casein significantly stimulated the proliferation of PMNs, from 1.6 % of total cell count in unincubated controls, to between 2.9 % and 14.9 % of total in cells incubated with Synthetic peptides derived from casein (Figure 7).

***Synthetic peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein stimulate Granulopoietic (GM) cell proliferation in CFU-GM:*** As mentioned hereinabove, formation and expansion of CFU-GM (Granulocyte and Monocyte) colonies, and CFU-GEMM (Granulocyte, Erythroid, Macrophage and Megakaryocyte) colonies constitute one of the early events in the differentiation of hematopoietic progenitor cells in the bone marrow. The effect of synthetic peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein on the proliferation of granulocytes and macrophages in murine primary bone marrow cells was demonstrated under the same conditions outlined for the stimulation of megakaryocytes, with the addition of cytokine IL-3 and granulocyte cell stimulating factor (G-CSF). Incubation of murine bone marrow progenitor cells with synthetic peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein representing amino acids 1-22 (J, SEQ ID No. 21) and 1-6 (30-4, SEQ ID No. 5), alone or in combination (Fig. 19) significantly stimulated the proliferation of granulocytes, when added along with G-CSF (18 % and 25 % increase for "30-4" and "J", respectively, in the presence of G-CSF)(Fig 19).

A similar effect of synthetic peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein was observed on the proliferation of granulocytes and macrophages from human bone marrow progenitor cells. Surprisingly, administration of synthetic peptides derived from  $\alpha$ -casein ("J", SEQ ID NO:21) or  $\beta$ -casein (SEQ ID NO:

28) enhanced the granulopoietic stimulating effects of G-CSF by >50% (100 µg "J") and 30% (300 µg "β"), respectively. (Fig. 20). Thus, synthetic peptides derived from αS1-, αS2-, β- or κ-casein or combinations thereof are effective in augmenting the effect of granulopoietic factors such as G-CSF on bone marrow hematopoietic progenitor cell differentiation and expansion.

***Peptides derived from natural casein stimulate hematopoiesis in vivo following irradiation and bone marrow transplant:*** Myeloablative therapy may lead to life-threatening reduction in thrombocytes and leukocytes, which may persist despite administration of blood cells and growth factors. The following demonstrates the effect of peptides derived from natural casein following irradiation and bone marrow transplantation.

***Peptides derived from natural casein enhance leukocyte and platelet reconstitution following syngeneic bone marrow transplantation in mice:*** When sub-lethally irradiated (600 cGy), minimally bone marrow-reconstituted, BALB/c mice (n = 12) received 1 mg per mouse peptides derived from natural casein via intravenous injection one day after bone marrow cell reconstitution, significant increases in peripheral white blood cell counts on days 4, 6 and 15 post-treatment were noted, compared to controls receiving human serum albumin (Figure 8). Platelet counts in the peripheral blood of both the treated and control irradiated, bone marrow transplanted mice were equally depressed up to 8 days post treatment. However, by the thirteenth day a clear advantage was noted for the mice treated with the peptides derived from natural casein, demonstrating a significant increase over the human serum albumin-treated controls which became even more pronounced by day 15 (Figure 9). Thus, peptides derived from natural casein enhance platelet and leukocyte reconstitution following transplantation with limiting numbers of bone marrow cells. It is expected that this effect will be further increased in reconstitution with optimal, rather than limiting numbers of bone marrow cells.

Further, in another series of similar experiments, it was observed that a partially purified (diafiltration with a 1 kDa cutoff membrane) preparation of



peptides derived from natural casein, comprising peptides derived from natural  $\alpha$ S1- and  $\beta$ -casein, significantly enhanced platelet reconstitution (by approx 25% over controls) in irradiated, bone marrow transplanted mice.

*Synthetic peptides derived from casein enhance leukocyte reconstitution following syngeneic bone marrow transplantation in mice:*  
When sub-lethally irradiated (600 cGy), minimally bone marrow-reconstituted, BALB/c mice ( $n = 5$  per synthetic peptide,  $n = 10$  in the control group) received 1 mg per mouse synthetic peptides (13-26 amino acids in length, see Table 3) derived from casein via an intraperitoneal injection one day after bone marrow transplantation, a clear enhancement of leukocyte reconstitution was observed. Significant increases in peripheral white blood cell counts over a 10 to 14 day period were noted with peptides having 15 (day 10:  $1.72 \times 10^6$  cells per ml; day 12:  $6.54 \times 10^6$  cells per ml) and 22 (day 10:  $2.74 \times 10^6$  cells per ml; day 12:  $5.20 \times 10^6$  cells per ml) amino acids (see Table 3), compared to controls receiving human serum albumin (day 10:  $1.67 \times 10^6$  cells per ml; day 12:  $4.64 \times 10^6$  cells per ml). Thus, synthetic peptides derived from casein enhance leukocyte reconstitution following transplantation with limiting numbers of bone marrow cells.

In a series of similar experiments, F1 mice ( $n = 5$  mice per group) which had been sub-lethally irradiated (750 cGy) and bone-marrow-reconstituted, as described above, received intravenous administration of 1mg of synthetic peptides derived from  $\alpha$ S1- (SEQ ID NO. 21),  $\beta$ - (SEQ ID NOs. 28), or  $\kappa$ -casein (SEQ ID NO: 434), alone or in combination, or peptides derived from natural  $\alpha$ S1- or  $\kappa$ -casein, one day following reconstitution. Peripheral white blood cell counts (Fig. 24) clearly demonstrate the strong stimulation of early leukocyte reconstitution (5 and 7 days post-transplantation) with both peptides derived from natural  $\alpha$ S1- and  $\kappa$ -casein, and synthetic peptides derived from  $\alpha$ S1-,  $\beta$ -, or  $\kappa$ -casein.

While reducing the present invention to practice, it was uncovered that a combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is significantly more

effective than the same amount of individual peptides. Mice treated with a combination of optimal doses of synthetic peptides derived from  $\alpha$ S1-(SEQ ID No. 21) and  $\beta$ -casein (SEQ ID No. 28) stimulated leukocyte reconstitution to a significantly greater degree than the individual component synthetic peptides  
5 derived from  $\alpha$ S1- or  $\beta$ -casein alone (Fig. 25).

***Synthetic peptides derived from casein enhance platelet reconstitution following syngeneic bone marrow transplantation in mice:*** In order to confirm the observed ability of synthetic peptides derived from casein to enhance megakaryocyte proliferation in hematopoietic stem cell cultures (see  
10 Figures 6 and 7), the peptides' effects on platelet reconstitution *in vivo* was investigated. When lethally irradiated (800 cGy), minimally bone marrow-reconstituted, mice (n = 5 per group) received 100  $\mu$ g per mouse synthetic peptides 4P and 3a (6 and 12 amino acids in length, respectively - see Table 3) in 4 daily intraperitoneal injections (4-7 days post-transplantation), a clear  
15 enhancement of platelet reconstitution over untreated controls was observed. Significant increases in platelet counts at 10 and 12 days post transplantation were noted for both peptides. Treatment with peptide 4P increased counts by 29 % ( $872 \times 10^3/\text{ml}$  compared with  $676 \times 10^3/\text{ml}$  in the control group) at 12 days post transplantation while treatment with peptide 3a increased counts by up to  
20 35.5 % ( $229 \times 10^3/\text{ml}$  compared with  $169 \times 10^3/\text{ml}$  in the control group) at 10 days, and up to 13.5 % ( $622 \times 10^3/\text{ml}$  compared with  $461 \times 10^3/\text{ml}$  in the control group) at 12 days post transplantation. Thus, the same synthetic peptides derived from casein enhance megakaryocyte proliferation *in vitro* and platelet reconstitution following bone marrow transplantation *in vivo*.

25 In an additional series of similar experiments, F1 mice sub-lethally irradiated (750 cGy) and minimally-bone marrow reconstituted ( $3 \times 10^6$  cells) which received intravenous administration of 1mg of synthetic peptides derived from casein demonstrated a significant increase in platelet counts. Mice receiving a synthetic peptide representing amino acids 193-208 of  $\beta$ -casein  
30 (SEQ ID NOs. 28), and the synthetic peptide representing amino acids 106- 127

of  $\kappa$ -casein (SEQ ID NO. 30) had enhanced platelet counts of 32% and 26% greater, respectively, compared to those of untreated control mice at 10 days post-transplantation. Bone marrow recipient mice, treated with synthetic peptide representing amino acids 1-22 of  $\alpha$ S1-casein (SEQ ID NO. 21) ("J"),  
5 showed similar enhancement of platelet reconstitution at 10 days post-transplantation (Figure 23).

***Peptides derived from natural casein inhibit in vitro infection of lymphocytic T cell lines by HIV-1 virus***

***Penetration of peptides derived from natural casein into lymphocytic***  
10 ***Tcells:*** In order to investigate the mechanisms of immune stimulatory and anti-viral effects of peptides derived from natural casein, susceptible Sup-T1 and CEM cultured human T-cells were treated with peptides derived from natural casein prior to *in vitro* infection with HIV-1 virus. Fluorescent microscopy revealed that FITC-conjugated peptides derived from natural casein (100  $\mu$ g per  
15 ml) penetrated the Sup-T1 cells when incubated therewith as described above (Figures 10a-f). A small amount of label was observed in the cytoplasm of the cells after 15 minutes (Figures 10a-b). At 30 minutes (Figures 10c-d) more label was observed in the cytoplasm, with limited nuclear uptake. From 1-hour incubation and on (Figures 10e-f), FITC-labeled peptides derived from natural  
20 casein were observed in the cytoplasm, but mostly they were concentrated in the cell nucleus. Analysis of the Sup-T1 cells by flow cytometry confirmed increasing uptake of the labeled peptides derived from natural casein from 5 minutes post incubation.

***Peptides derived from natural casein enhance human lymphocyte***  
25 ***proliferation:*** The presence of peptides derived from natural casein in the culture medium resulted in increased Sup-T1 cell counts over a period of 14 days. The greatest increases in cell number at 7 days was observed for 50  $\mu$ g per ml peptides derived from natural casein (42 %), for 1000  $\mu$ g at 10 days (30 %) and for 600  $\mu$ g (32 %) at 14 days incubation (data not shown).

Measurement of [ $^3\text{H}$ ]-thymidine incorporation by the cultured cells, providing a proliferation index, reflected the increase in cell number, with the most significant effect noted for 600  $\mu\text{g}$  per ml peptides derived from natural casein on day 10 and 50  $\mu\text{g}$  per ml on day 14 (Figure 11). The reduced proliferation indices at 14 days probably reflect cell overgrowth and nutrient depletion.

***Synthetic peptides derived from casein enhance human lymphocyte proliferation:*** The presence of synthetic peptides derived from casein (all peptides listed in Table 3) in the culture medium resulted in increased Sup-T1 cell counts over a period of 10 days. The increase was similar for all synthetic peptides. The greatest increases in lymphocyte cell number in infected cells were observed for 250  $\mu\text{g}$  and 500  $\mu\text{g}$  per ml of peptide representing the first 9 amino acids (80 % and 33 %, respectively) (data not shown).

***Peptides derived from natural casein inhibit HIV-1 infection in human lymphocyte cells:*** Susceptible CEM lymphocyte cells pretreated with peptides derived from natural casein (50-1000  $\mu\text{g}$  per ml) 24 or 48 hours prior to incubation with HIV-1, or exposed to HIV-1 pretreated 3 hours with peptides from natural casein, exhibited enhanced cell proliferation and reduced levels of viral infection compared to untreated controls. Cell counts and HIV-1 p $^{24}$  antigen assay at 15 days post infection revealed 100 % inhibition of viral infection after 3 hours incubation of viruses with 600-1000  $\mu\text{g}$  per ml peptides derived from natural casein and 98 % and 99 % inhibition after 24 hours incubation of cells with 50 and 600  $\mu\text{g}$  per ml peptides, respectively (comparing cell numbers with uninfected controls UIF). Longer incubation times were not found to be more effective (Figure 12). Although increasing concentrations of peptides derived from natural casein enhanced cell proliferation at 3 and 24 hours post infection, viral infection is most significantly inhibited in these fastest growing cultures. An even more dramatic enhancement of cell proliferation and inhibition of HIV-1 infection was observed in Sup-T1 cells pretreated with peptides derived from natural casein before HIV-1 infection

(average inhibition of viral infection of 96.7 %, 88.7 % and 95.7 % for 3 hours pretreatment of virus, and 24 hours and 48 hours pretreatment of cells, respectively) (not shown). Thus, peptides derived from natural casein penetrate human cultured lymphocyte cells and their nuclei, enhance cell growth, and significantly reduce the susceptibility of CD4 cells to HIV-1 infection. As such, peptides derived from natural casein are expected to be useful both at preventing HIV infection and for post infection treatment of HIV infected and AIDS patients.

*Synthetic peptides derived from casein inhibit HIV-1 infection in human lymphocyte cells:* The ability of synthetic peptides derived from casein to inhibit HIV-1 infection in human lymphocyte cells was demonstrated using CEM-lymphocyte cells under the same conditions outlined above. Susceptible CEM lymphocyte cells pretreated with synthetic peptides derived from  $\alpha$ S1-casein (50-1000  $\mu$ g per ml) 24 or 48 hours prior to incubation with HIV-1, or exposed to HIV-1 pretreated 3 hours with synthetic peptides from  $\alpha$ S1-casein, exhibited enhanced cell proliferation and reduced levels of viral infection compared to untreated controls.,24 or 48 hours incubation with synthetic peptides representing the first 3 amino acids of  $\alpha$ S1casein conferred a significant degree of resistance to infection following incubation withHIV-1. Lymphocyte cell numbers were  $1.29 \times 10^6$  (100 $\mu$ g per ml) and  $2.01 \times 10^6$  (500 $\mu$ g per ml) in the treated cells as compared to the infected HIV-1 control of  $1.06 \times 10^6$  (Figure 13). HIV-1 infection levels in the same cells, measured by the HIV-P<sup>24</sup> antigen assay at 7 days post infection, was significantly reduced in the peptide treated cells (0.17 and 0.14ng P<sup>24</sup> Antigen/ml with100 $\mu$ g/ml and 500 $\mu$ g/ml respectively), as compared to the untreated controls (0.52 ng P<sup>24</sup> Ag/ ml).

Likewise, significant inhibition of HIV-1 infection was observed in the CEM cells exposed to viruses that had been pre-treated (3 hours) with the synthetic casein derived peptide representing the first 5 amino acids of  $\alpha$  S1casein.

Cell counts in the cultures incubated with 10 and 25 $\mu$ g peptide 3P per ml were  $1.17 \times 10^6$  and  $1.26 \times 10^6$  respectively, as compared to the infected HIV-1 control of  $1.06 \times 10^6$ .

5 HIV- P<sup>24</sup> antigen assay at 7 days post infection, revealed significant reduction in HIV-1 infection levels in treated cultures (0.26 and 0.18ng P<sup>24</sup> Ag per ml for 10 and 25 $\mu$ g per ml respectively, as compared to the control of 0.52 ng P<sup>24</sup> Ag per ml).

10 Likewise, 3 hours preincubation of the virus with the synthetic peptide derived from casein 4P, representing the first 6 amino acids of  $\alpha$ S1casein had a significant effect on the susceptibility of CEM lymphocyte cells to infection with HIV-1.

Cell numbers were most affected at concentrations of 25 and 250 $\mu$ g per ml ( $1.26 \times 10^6$ , and  $1.59 \times 10^6$  respectively, as compared to the infected control value of  $1.06 \times 10^6$ ).

15 Assay of HIV-P<sup>24</sup> antigen at 7 days post infection, revealed a dose dependent reduction in viral particles as compared to the untreated, infected control cultures (Figure 13). Thus, the protection from HIV-1 infection afforded lymphocyte cells by the peptides derived from natural casein is retained in synthetic peptides derived from casein representing as few as the first five N-terminal amino acids of  $\alpha$ S-1 casein.

20 *Peptides derived from natural casein prevent development of glucosuria in Non-Obese Diabetic (NOD) mice:* Non-Obese Diabetic (NOD) mice spontaneously develop Juvenile (Type I, IDDM) Diabetes, an autoimmune condition causing inflammation of the pancreatic  $\beta$  cells and ending in disease and death. Female NOD mice are extremely susceptible, demonstrating  
25 evidence of macrophage invasion of the pancreatic islet interstitial matrix as early as 5 weeks old. A once or twice weekly injection of 100  $\mu$ g peptides derived from natural casein for 5 weeks (5 or 10 injections total) were completely effective in preventing the glucosuria associated with the onset and

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course of the disease. By 200 days 100 % of the untreated control mice (n = 5) had become diabetic, and subsequently died, while the treated mice (n = 10) remained 100 % euglycemic, all still surviving at 365 days (Figure 14). Thus, peptides derived from natural casein effectively protected genetically susceptible mice against the onset of this autoimmune inflammatory condition.

***Synthetic peptides derived from casein prevent development of glucosuria in Non-Obese Diabetic (NOD) mice:***

The preventative effect of synthetic peptides derived from casein on the development of glucosuria in NOD mice was demonstrated under the same conditions outlined above, except that the mice were injected only once weekly for five (5) weeks with 100 µg of synthetic peptides derived from casein. The results of these experiments are presented in Table 4 below:

TABLE 4

***The effect of synthetic peptides on IDDM in NOD mice***

Peptide Derivative code	Healthy/Total*	Urine Sugar	IPGT TEST	
			0 min. (pre- load)	60 min. post load
Y(SEQ ID NO:7)	1/5	Negative	121	138
X(SEQ ID NO:8)	3/5	Negative	94	114
		Negative	104	119
		Negative	141	114
1a(SEQ ID NO:9)	1/5	Negative	88	106
2a(SEQ ID NO:10)	4/5	Negative	216	183
		Negative	112	119
		Negative	95	107
		Negative	169	204
3a(SEQ ID NO:11)	3/5	Negative	135	137
		Negative	205	197
		Negative	201	211
A(SEQ ID NO:12)	2/5	Negative	134	164
		Negative	105	107

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B(SEQ ID NO:13)	2/5	Negative	130	117
		Negative	130	97
D(SEQ ID NO:15)	2/5	Negative	99	108
		Negative	130	136
I(SEQ ID NO:20)	2/5	Negative	324	not tested
		Negative	124	138
J(SEQ ID NO:21)	3/5	Negative	166	not tested
		Negative	193	not tested
		Negative	186	not tested
K(SEQ ID NO:22)	2/5	Negative	116	143
		Negative	443	not tested
Chay-13	2/5	Negative	123	130
		Negative	111	111
Chay-13	2/5	Negative	128	116
		Negative	113	125
Control	0/5			

Blood was drawn from the paraorbital plexus at 0 min and 60 min after the intraperitoneal injection of glucose 1 g/kg body weight. Plasma glucose levels were determined with a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA) and expressed as mmol/L.

5 \* Healthy and well = Sugar not detected in urine.

Glucosuria = > 1000 mg/dL.

IPGTT performed with 6 healthy female control mice: 0 min- 110 mmol/L;

60 min - 106 mmol/L blood glucose.

10 The synthetic peptides derived from casein representing the first 9 (X) (SEQ ID NO. 8), 11 (2a) (SEQ ID NO. 10) and 12 (3a) (SEQ ID NO. 11) amino acids and higher chain length of  $\alpha$ S1 casein, were highly effective in preventing the glucosuria associated with the onset and course of the disease.

15 Effect of treatment with synthetic peptides derived from casein was evaluated after 25 weeks. At that time, all 5 mice in the untreated control group (n = 5) had become diabetic, as indicated by the presence of frank (>1000 mg/dl) glucosuria (Table 4).

No glucosuria was detected in three of the five (3/5) NOD mice treated with the synthetic peptide representing the first nine (9) amino acids from the N



terminal of  $\alpha$ S1 casein. Of the group injected with the synthetic peptide of eleven (11) amino acids from the N terminal of  $\alpha$ S1 casein, no glucosuria was detected in four out of five (4/5) of the NOD mice

5 In the groups of peptide treated mice in which glucosuria was detected, the onset was generally significantly delayed (by 3- 5 weeks) relative to the onset of glucosuria in untreated controls (data not shown), indicating a clearly protective effect of the peptides even when incomplete.

The protective effects of shorter synthetic peptides derived from casein have also been studied in NOD mice. In an additional series of experiments  
10 similar to the abovementioned, administration of peptides representing the first 3 (1P) and 4 (2P) N-terminal amino acids of  $\alpha$ S1 casein effectively prevented the onset of glucosuria in the treated mice (assayed at week 16), while the untreated controls had all become diabetic (100 % glucosuria) (data not shown).

The glucose tolerance (IPGT) test performed after 25 weeks with the  
15 healthy and well NOD mice, of the group injected with the synthetic casein derived peptide of the first 9 amino acids (SEQ ID NO. 8), showed no evidence of abnormal glucose metabolism (normal glycemic values pre- and 60 minutes post- glucose loading).

In the group treated with the synthetic peptide derived from casein  
20 representing the first 11 amino acids of the N-terminal of  $\alpha$ S1 casein (2a) (SEQ ID NO. 10), resting plasma glucose levels were somewhat elevated in two of the five mice (215 and 159 mmol/L), and remained mildly elevated at (183 and 204 mmol/L) 60 minutes post load, indicating mild diabetic tendencies. The other two mice remained within normal glycemic range throughout the test  
25 (Table 4). In general, the normal results of the IPGTT reflected the absence of glucosuria in the healthy, surviving peptide-treated mice (Table 4). Thus, synthetic peptides representing only a few amino acids from the N-terminal of  $\alpha$ S 1 casein, as well as peptides derived from native casein dramatically reduce

the susceptibility of genetically predisposed NOD mice to onset of autoimmune diabetic disease.

***Synthetic casein- derived peptides significantly reduce Total Cholesterol blood levels (TC), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL):*** Intraperitoneal administration of Synthetic peptides derived from casein caused a significant reduction in the blood lipid (HDL, LDL and TC) values in experimentally hypercholesterolemic mice. After one week of the atherogenic Thomas Hartroft diet, the blood cholesterol levels of the mice had risen to the levels of 318mg/dl.

One week post treatment with 1 mg synthetic peptides derived from casein per mouse, the group treated with the Synthetic peptides derived from casein representing the first 5 (3P) (SEQ ID NO. 4) and 11 (2a) (SEQ ID NO. 10) amino acids of  $\alpha$ S1 casein, had significantly reduced TC, HDL and LDL values, compared to those of the control group [TC: 308 and 279mg/dl respectively; HDL: 42.5 mg/dl and 41mg/dl respectively and LDL: 247mg/dl and 221mg/dl respectively as compared to 393mg/dl (TC), 54.5 mg/dl (HDL) and 326 mg/dl (LDL) in the diet-induced hypercholesterol-/hyperlipidemic control group] (Figure 15). Thus, synthetic peptides representing the first few N-terminal amino acids of  $\alpha$ S 1 casein effectively reduced experimentally induced hyperlipidemia and hypercholesterolemia within 1 week after a single, intraperitoneal administration.

***Clinical trials with peptides derived from natural casein:***

Patients received a series of one, two or three intramuscular injections of 50 mg peptides derived from natural casein each, divided into three depots each treatment, as indicated.

***Peptides derived from natural casein stimulates hematopoiesis in cancer patients:*** The hematology profiles of six cancer patients who had received or were receiving chemotherapy were examined before and following administration of peptides derived from natural casein, as indicated. Special attention was paid to changes in the Platelet (PLT), Leukocyte (WBC),

Erythrocyte (RBC) and Hemoglobin (HGB) values, representing thrombocytopoiesis, leukocytopoiesis, and erythrocytopoiesis, respectively.

*G.T., (Female patient, Patient 1):* Patient had ovarian cancer, undergone a hysterectomy followed by chemotherapy. She received two  
5 intramuscular injections of peptides derived from natural casein at two and then two and one half months post operation. No chemotherapy was administered between the first and second administrations of peptides derived from natural casein. Blood tests from 6 days post first injection, 7, and 13 days post second injection reflect a considerable increase in platelet and WBC components, as  
10 well as increased RBC (Figure 16).

*E.C., (Female patient, Patient 2):* Patient underwent a radical mastectomy for lobular carcinoma in 1983, and six years later suffered from gastric metastases. Three days prior to commencement of chemotherapy, she received one intramuscular injection (in three depots) of peptides derived from  
15 natural casein by injection, and a second 10 days after the chemotherapy. Although the blood counts from 10 and 16 days post chemotherapy indicated an attenuation of the depressed hematological profile usually encountered following chemotherapy, the most significant effects of peptides derived from natural casein were noted 3 days after the first injection, prior to the  
20 chemotherapy (Figure 16).

*E.S., (Female patient, Patient 3):* Patient was suffering from widespread metastatic dissemination of a breast carcinoma first discovered in 1987. Two years later, she received a first intramuscular injection of peptides derived from natural casein, and a second 23 days later. No additional therapy  
25 was administered during this period. Blood tests indicate a strong enhancement of PLT seven days after the first treatment and a significant increase in RBC and WBC seven days after the second treatment (Figure 16).

*J.R., (Female patient, Patient 4):* Patient's diagnosis is breast cancer with bone metastases. She received one intramuscular injection of peptides  
30 derived from natural casein 8 days before commencing chemotherapy, and

another, 14 days later. The most significant effect is clearly seen in the rapid return of WBC levels following chemotherapy-induced depression (Figure 16).

**D.M., (Female patient, Patient 5):** Patient suffering from hepatic cancer with widespread metastatic dissemination. She received three intramuscular injections of peptides derived from natural casein at 10, 8 and 6 days before receiving chemotherapy. A second series of injections was initiated 10, 12 and 14 days following the chemotherapy treatment. Although a significant effect on the hematological profile is noted following the first series of injections and prior to the chemotherapy, the most dramatic improvements are seen in the rapid return of depressed post-chemotherapy values to normalized cell counts following the second series of peptides derived from natural casein injections (Figure 16).

Thus, administration of peptides derived from natural casein to cancer patients results in improved hematological profiles, specifically enhanced erythropoiesis, leukocytopoiesis and thrombocytopoiesis, and is capable of moderating and shortening the duration of chemotherapy-induced depression of blood components.

**Peptides derived from natural casein stimulates thrombocytopoiesis in transplant recipients with resistant thrombocytopenia:** Prolonged transfusion-resistant thrombocytopenia with episodes of severe bleeding, may be a life threatening complication of bone marrow transplantation, especially where traditional therapies are ineffective. Two patients with severe resistant thrombocytopenia were treated with peptides derived from natural casein.

**M-1 (Female patient):** 32 year old patient suffering from Acute Myeloid Leukemia in complete remission, following autologous stem cell transplantation. She had experienced two life-threatening bleeding episodes, involving pulmonary hemorrhage and a large obstructive hematoma in the soft palate. At more than 114 days post transplantation, platelet counts were refractive to rhIL-3, rhIL-6, intravenous gamma globulin, and recombinant erythropoietin. Following two intra muscular treatments of 50 mg peptides

derived from natural casein (each treatment divided into three depots), her condition improved immediately. Along with the rapid return of normal platelet counts (Figure 17), her distal limb bleeding with exertion and petechiae subsided, she was able to resume walking, and returned to her home overseas with no complications or side effects.

**M-2 (Male patient):** 30 year old patient suffering from Acute Myeloid Leukemia in a second complete remission following autologous stem cell transplantation, exhibiting totally resistant platelet counts and massive gastrointestinal bleeding episodes. He required daily transfusions of packed cells, had developed hypoalbuminemia, and failed to respond to extensive therapy with rIL-3, rIL-6 and gamma globulin. Following two intramuscular treatments, each of 50 mg peptides derived from natural casein in three depots 86 days post transplantation, rapid platelet reconstitution (Figure 18) and gradual discontinuation of the bleeding was observed. No further treatment was required, and the patient is presently completely asymptomatic with normal platelet count.

Thus, one course of two intramuscular injections of peptides derived from natural casein at 0.7- 1.0 mg per kg body weight, each divided into three depots, was effective in rapidly reconstituting platelet counts and diminishing associated clinical symptoms in patients suffering from prolonged, transfusion resistant thrombocytopenia with life-threatening bleeding episodes.

***Peptides derived from natural casein decreases triglycerides and Total Cholesterol in familial hyperlipidemia:***

**M.S. (Female patient):** Patient is a 38 year old female with family history of hyperlipidemia. Before treatment with peptides derived from natural casein, blood chemistry profile revealed elevated total cholesterol (321 mg per dl), triglycerides (213 mg per dl; normal range 45 – 185 mg per dl) and elevated LDL-cholesterol (236.4 mg per dl; normal range 75 – 174 mg per dl). One month after a single administration of 50 mg peptides derived from natural casein (in three intra muscular depots) the hyperlipidemia was stabilized: total

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cholesterol was reduced to 270 mg per dl, triglycerides were 165 mg per dl and LDL-cholesterol was 201 mg per dl, still higher than normal range but significantly reduced from the pretreatment value. No additional treatment was administered. Thus, treatment with peptides derived from natural casein is effective in rapidly bringing about a significant reduction in otherwise untreated hyperlipidemia in humans.

***Peptides derived from natural casein stimulate normoglobinemia in a case of occult bleeding:***

**D. G. (Male patient):** Patient is a 75 year old male suffering from anemia and hypoglobinemia (depressed RBC, HGB, HCT, MCH and MCHC) associated with extensive occult bleeding. One month after receiving one intramuscular injection of 50 mg peptides derived from natural casein (in three depots), a significant reduction of the anemia was observed. After two months, RBC approached normal values (4.32 instead of 3.44 M per  $\mu$ l), HGB increased (11.3 instead of 8.9 g per dl) and HCT, MCH and MCHC all improved to nearly normal values, despite the persistence of occult bleeding. Thus, one injection of peptides derived from natural casein seemed capable of stimulating erythropoiesis and reducing anemia associated with blood loss in humans.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

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Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents, patent applications and sequences identified by an accession number, mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent, patent application or sequence was specifically and individually indicated to be incorporated herein by reference. In addition,

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citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.



**WHAT IS CLAIMED IS:**

1. A method of preventing or treating an autoimmune or infectious disease or condition, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.
2. The method of claim 1, wherein said autoimmune or infectious disease or condition is selected from the group consisting of a viral disease, a viral infection, AIDS, and infection by HIV.
3. The method of claim 1, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.
4. The method of claim 1, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.
5. The method of claim 1, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.
6. The method of claim 1, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.
7. The method of claim 1, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.
8. The method of claim 7, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs:

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1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID NOs: 1-33 and 434-4000.

9. A method of preventing or treating a blood disease or condition, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

10. The method of claim 9, wherein said blood disease or condition is selected from the group consisting of thrombocytopenia, pancytopenia, granulocytopenia, an erythropoietin treatable condition, and a thrombopoietin treatable condition.

11. The method of claim 9, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

12. The method of claim 9, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

13. The method of claim 9, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

14. The method of claim 9, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

15. The method of claim 9, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

16. The method of claim 15, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

17. The method of claim 9, further comprising administering to said subject in need thereof an effective amount of a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

18. A method of modulating blood cell formation, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

19. The method of claim 18, wherein said modulating blood cell formation is selected from the group consisting of inducing hematopoiesis, inducing hematopoietic stem cells proliferation, inducing hematopoietic stem cells proliferation and differentiation, inducing megakaryocytopoiesis, inducing erythropoiesis, inducing leukocytopoiesis, inducing thrombocytopoiesis, inducing plasma cell proliferation, inducing dendritic cell proliferation and inducing macrophage proliferation.

20. The method of claim 18, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

21. The method of claim 18, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

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22. The method of claim 18, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.
23. The method of claim 18, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.
24. The method of claim 18, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.
25. The method of claim 24, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.
26. The method of claim 18, further comprising administering to said subject in need thereof an effective amount of a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).
27. A method of enhancing peripheral stem cell mobilization, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.
28. The method of claim 27, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

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29. The method of claim 27, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

30. The method of claim 27, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

31. The method of claim 27, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

32. The method of claim 27, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

33. The method of claim 32, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

34. The method of claim 27, further comprising administering to said subject in need thereof an effective amount of a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

35. A method of preventing or treating a metabolic disease or condition, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

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36. The method of claim 35, wherein said metabolic disease or condition is selected from the group consisting of NIDDM, IDDM, glucosuria, hyperglycemia, hyperlipidemia, and hypercholesterolemia.

37. The method of claim 35, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

38. The method of claim 35, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

39. The method of claim 35, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

40. The method of claim 35, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

41. The method of claim 35, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

42. The method of claim 41, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

43. A method of preventing or treating conditions associated with myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT), the method comprising administering to a

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subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

44. The method of claim 43, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

45. The method of claim 43, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

46. The method of claim 43, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

47. The method of claim 43, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

48. The method of claim 43, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

49. The method of claim 48, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

50. The method of claim 43, further comprising administering to said subject in need thereof an effective amount of a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

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51. A method of augmenting the effect of a blood cell stimulating factor, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

52. The method of claim 51, wherein said blood cell stimulating factor is selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

53. The method of claim 51, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

54. The method of claim 51, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

55. The method of claim 51, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

56. The method of claim 51, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

57. The method of claim 51, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

58. The method of claim 57, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.



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59. The method of claim 51, further comprising administering to said subject in need thereof an effective amount of erythropoietin, thrombopoietin or granulocyte colony stimulating factor (G-CSF).

60. A method of enhancing colonization of donated blood stem cells in a myeloablated recipient, the method comprising treating a donor of said donated blood stem cells with a therapeutically effective amount of peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof prior to donation and implanting the donated blood stem cells in the recipient.

61. The method of claim 60, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

62. The method of claim 60, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

63. The method of claim 60, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

64. The method of claim 60, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

65. The method of claim 60, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

66. The method of claim 65, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs:

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1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

67. The method of claim 60, further comprising treating said donor with a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF) prior to donation and implanting the blood stem cells in the recipient.

68. A method of enhancing colonization of donated blood stem cells in a myeloablated recipient, the method comprising treating said donated blood stem cells with a therapeutically effective amount of peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof prior to implanting the donated blood stem cells in the recipient.

69. The method of claim 68, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

70. The method of claim 68, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

71. The method of claim 68, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

72. The method of claim 68, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

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73. The method of claim 68, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

74. The method of claim 73, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

75. The method of claim 68, further comprising treating said donated blood cells with a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF) prior to implanting the blood stem cells in the recipient.

76. A method of enhancing colonization of blood stem cells in a myeloablated recipient, the method comprising treating said blood stem cells with a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof prior to implanting the blood stem cells in the recipient.

77. The method of claim 76, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

78. The method of claim 76, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

79. The method of claim 76, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

80. The method of claim 76, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

81. The method of claim 76, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

82. The method of claim 81, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

83. The method of claim 76, further comprising treating said blood stem cells with a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF) prior to implanting the blood stem cells in the recipient.

84. A method for preventing or treating a condition associated with a SARS infective agent, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

85. The method of claim 84, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

86. The method of claim 84, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

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87. The method of claim 84, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

88. The method of claim 84, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

89. The method of claim 84, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

90. The method of claim 89, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

91. The method of claim 84, wherein said SARS infective agent is a coronavirus.

92. The method of claim 91, wherein said coronavirus is SARS-CoV.

93. A method for preventing or treating a bacterial disease or condition, the method comprising administering to a subject in need thereof a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof.

94. The method of claim 93, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

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95. The method of claim 93, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

96. The method of claim 93, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

97. The method of claim 94, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

98. The method of claim 94, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

99. The method of claim 98, wherein said chimeric peptide comprises a first  $\alpha$ S1-casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

100. A pharmaceutical composition for preventing or treating an autoimmune or infectious disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

101. The of claim 100, wherein said autoimmune or infectious disease or condition is selected from the group consisting of a viral disease, a viral infection, AIDS, and infection by HIV.

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102. The pharmaceutical composition of claim 100, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

103. The pharmaceutical composition of claim 100, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

104. The pharmaceutical composition of claim 100, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

105. The pharmaceutical composition of claim 100, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

106. The pharmaceutical composition of claim 100, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

107. The pharmaceutical composition of claim 106, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

108. A pharmaceutical composition for preventing or treating a blood disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

109. The pharmaceutical composition of claim 108, wherein said blood disease or condition is selected from the group consisting of thrombocytopenia, pancytopenia, granulocytopenia, an erythropoietin treatable condition, and a thrombopoietin treatable condition and a granulocyte colony stimulating factor treatable condition.

110. The pharmaceutical composition of claim 108, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

111. The pharmaceutical composition of claim 108, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

112. The pharmaceutical composition of claim 108, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

113. The pharmaceutical composition of claim 108, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

114. The pharmaceutical composition of claim 108, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

115. The pharmaceutical composition of claim 114, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set



forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

116. The pharmaceutical composition of claim 108, further comprising, as an active ingredient, a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

117. A pharmaceutical composition for modulating blood cell formation, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

118. The pharmaceutical composition of claim 117, wherein said modulating blood cell formation is selected from the group consisting of inducing hematopoiesis, inducing hematopoietic stem cells proliferation, inducing hematopoietic stem cells proliferation and differentiation, inducing megakaryocytopoiesis, inducing erythropoiesis, inducing leukocytopoiesis, inducing thrombocytopoiesis, inducing granulocytopoiesis, inducing plasma cell proliferation, inducing dendritic cell proliferation and inducing macrophage proliferation.

119. The pharmaceutical composition of claim 117, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

120. The pharmaceutical composition of claim 117, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

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121. The pharmaceutical composition of claim 117, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

122. The pharmaceutical composition of claim 117, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

123. The pharmaceutical composition of claim 117, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

124. The pharmaceutical composition of claim 123, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

125. The pharmaceutical composition of claim 117, further comprising, as an active ingredient, a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

126. A pharmaceutical composition for enhancing peripheral stem cell mobilization, the pharmaceutical composition comprising, as an active ingredient, a therapeutically effective amount of a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

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127. The pharmaceutical composition of claim 126, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

128. The pharmaceutical composition of claim 126, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

129. The pharmaceutical composition of claim 126, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

130. The pharmaceutical composition of claim 126, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

131. The pharmaceutical composition of claim 126, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

132. The pharmaceutical composition of claim 131, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

133. The pharmaceutical composition of claim 126, further comprising, as an active ingredient, a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

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134. A pharmaceutical composition for preventing or treating a metabolic disease or condition, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

135. The pharmaceutical composition of claim 134, wherein said metabolic disease or condition is selected from the group consisting of NIDDM, IDDM, glucosuria, hyperglycemia, hyperlipidemia, and hypercholesterolemia.

136. The pharmaceutical composition of claim 134, wherein said peptide derived from  $\alpha$ S1 casein is a synthetic peptide.

137. The pharmaceutical composition of claim 134, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

138. The pharmaceutical composition of claim 134, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

139. The pharmaceutical composition of claim 135, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

140. The pharmaceutical composition of claim 135, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

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141. The pharmaceutical composition of claim 140, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

142. A pharmaceutical composition for preventing or treating conditions associated with myeloablative doses of chemoradiotherapy supported by autologous bone marrow or peripheral blood stem cell transplantation (ASCT) or allogeneic bone marrow transplantation (BMT), the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

143. The pharmaceutical composition of claim 142, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

144. The pharmaceutical composition of claim 142, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

145. The pharmaceutical composition of claim 142, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

146. The pharmaceutical composition of claim 142, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

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147. The pharmaceutical composition of claim 142, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

148. The pharmaceutical composition of claim 147, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

149. The pharmaceutical composition of claim 142, further comprising, as an active ingredient, a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

150. A pharmaceutical composition for augmenting the effect of a blood cell stimulating factor, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

151. The pharmaceutical composition of claim 150, wherein said blood cell stimulating factor is selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

152. The pharmaceutical composition of claim 150, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

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153. The pharmaceutical composition of claim 150, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

154. The pharmaceutical composition of claim 150, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

155. The pharmaceutical composition of claim 150, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

156. The pharmaceutical composition of claim 150, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

157. The pharmaceutical composition of claim 156, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

158. The pharmaceutical composition of claim 150, further comprising, as an active ingredient thrombopoietin, erythropoietin or granulocyte colony stimulating factor (G-CSF).

159. A pharmaceutical composition for enhancing colonization of donated blood stem cells in a myeloablated recipient, the pharmaceutical composition comprising, as active ingredients, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

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160. The pharmaceutical composition of claim 159, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

161. The pharmaceutical composition of claim 159, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

162. The pharmaceutical composition of claim 159, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

163. The pharmaceutical composition of claim 159, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

164. The pharmaceutical composition of claim 159, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

165. The pharmaceutical composition of claim 164, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

166. The pharmaceutical composition of claim 159, further comprising, as an active ingredient thrombopoietin, erythropoietin or granulocyte colony stimulating factor (G-CSF).



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167. A pharmaceutical composition for enhancing colonization of blood stem cells in a myeloablated recipient, the pharmaceutical composition comprising as active ingredients, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

168. The pharmaceutical composition of claim 167, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

169. The pharmaceutical composition of claim 167, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

170. The pharmaceutical composition of claim 167, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

171. The pharmaceutical composition of claim 167, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

172. The pharmaceutical composition of claim 167, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

173. The pharmaceutical composition of claim 172, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

174. The pharmaceutical composition of claim 167, further comprising, as an active ingredient thrombopoietin, erythropoietin or granulocyte colony stimulating factor (G-CSF).

175. A pharmaceutical composition for treating or preventing an indication selected from the group consisting of autoimmune disease or condition, viral disease, viral infection, hematological disease, hematological deficiencies, thrombocytopenia, pancytopenia, granulocytopenia, hyperlipidemia, hypercholesterolemia, glucosuria, hyperglycemia, diabetes, AIDS, HIV-1, helper T-cell disorders, dendrite cell deficiencies, macrophage deficiencies, hematopoietic stem cell disorders including platelet, lymphocyte, plasma cell and neutrophil disorders, pre-leukemic conditions, leukemic conditions, immune system disorders resulting from chemotherapy or radiation therapy, human immune system disorders resulting from treatment of diseases of immune deficiency and bacterial infections, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

176. The pharmaceutical composition of claim 175, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

177. The pharmaceutical composition of claim 175, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

178. The pharmaceutical composition of claim 175, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

179. The pharmaceutical composition of claim 175, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

180. The pharmaceutical composition of claim 175, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

181. The pharmaceutical composition of claim 180, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

182. The pharmaceutical composition of claim 175, further comprising, as an active ingredient, a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

183. A pharmaceutical composition for treating or preventing an indication selected from the group consisting of hematological disease, hematological deficiencies, thrombocytopenia, pancytopenia, granulocytopenia, dendrite cell deficiencies, macrophage deficiencies, hematopoietic stem cell disorders including platelet, lymphocyte, plasma cell and neutrophil disorders, pre-leukemic conditions, leukemic conditions, myelodysplastic syndrome, non-myeloid malignancies, aplastic anemia and bone marrow insufficiency, the pharmaceutical composition comprising, as active ingredients, a blood cell stimulating factor and a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

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184. The pharmaceutical composition of claim 183, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

185. The pharmaceutical composition of claim 183, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

186. The pharmaceutical composition of claim 183, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

187. The pharmaceutical composition of claim 183, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

188. The pharmaceutical composition of claim 183, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

189. The pharmaceutical composition of claim 188, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

190. The pharmaceutical composition of claim 183, wherein said blood cell stimulating factor is selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

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191. A purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1- 33.

192. A purified chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

193. The chimeric peptide of claim 192 comprising a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

194. A pharmaceutical composition comprising a purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-33 and a pharmaceutically acceptable carrier.

195. A pharmaceutical composition comprising a purified chimeric peptide, said chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage, and a pharmaceutically acceptable carrier.

195. The pharmaceutical composition of claim 195, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

196. A pharmaceutical composition comprising a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF), in combination with a purified peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-33 and a pharmaceutically acceptable carrier.

197. A pharmaceutical composition comprising a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF), in combination with a purified chimeric comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

198. The pharmaceutical composition of claim 197, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

199. A pharmaceutical composition for preventing or treating a condition associated with a SARS infective agent, the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

200. The pharmaceutical composition of claim 199, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

201. The pharmaceutical composition of claim 199, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

202. The pharmaceutical composition of claim 199, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

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203. The pharmaceutical composition of claim 199, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

204. The pharmaceutical composition of claim 199, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

205. The pharmaceutical composition of claim 204, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000.

206. The pharmaceutical composition of claim 199, further comprising, as an active ingredient, a blood cell stimulating factor, said blood cell stimulating factor selected from the group consisting of thrombopoietin, erythropoietin and granulocyte colony stimulating factor (G-CSF).

207. The pharmaceutical composition of claim 199, wherein said SARS infective agent is a coronavirus.

208. The pharmaceutical composition of claim 207, wherein said coronavirus is SARS-CoV.

209. A pharmaceutical composition for preventing or treating a bacterial infection the pharmaceutical composition comprising, as an active ingredient, a peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein or combination thereof and a pharmaceutically acceptable carrier.

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210. The pharmaceutical composition of claim 209, wherein said peptide is a fragment derived from the N terminus portion of  $\alpha$ S1 casein by fragmentation of  $\alpha$ S1 casein.

211. The pharmaceutical composition of claim 209, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a synthetic peptide.

212. The pharmaceutical composition of claim 209, wherein said peptide derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein has a sequence as set forth in one of SEQ ID NOs: 1-33.

213. The pharmaceutical composition of claim 209, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a mixture of peptides.

214. The pharmaceutical composition of claim 209, wherein said combination of peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein is a chimeric peptide comprising at least two peptides derived from  $\alpha$ -,  $\beta$ - or  $\kappa$ -casein in covalent linkage.

215. The pharmaceutical composition of claim 214, wherein said chimeric peptide comprises a first  $\alpha$ S1 casein peptide having a sequence as set forth in one of SEQ ID NOs: 1-25 covalently linked to a second casein peptide having a sequence as set forth in any of SEQ ID Nos: 1-33 and 434-4000

216. A method of low-temperature processing of casein proteolytic hydrolysate, the method comprising:



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- a) obtaining a casein proteolytic hydrolysate comprising proteolytic enzymes;
  - b) cooling said casein proteolytic hydrolysate so as to inactivate said proteolytic enzymes;
  - c) adjusting the pH of said casein protein hydrolysate to an acid pH;
  - d) filtering said acidic casein protein hydrolysate, collecting the filtrate, and further acidifying said filtrate so as to precipitate proteins derived from natural casein;
  - e) separating and collecting said precipitate;
  - f) adjusting the pH of said precipitate to an alkaline pH so as to irreversibly inactivate said proteolytic enzymes; and
  - g) adjusting the pH of said precipitate to pH 7-9;
- thereby processing said casein protein hydrolysate at low temperature.

217. The method of claim 216, wherein step b comprises cooling to about 10°C.

218. The method of claim 216, wherein said adjusting said pH of step c comprises addition of acid to 2% (w/v) acid, and whereas said further acidifying said filtrate of step d comprises additional addition of acid to about 10% (w/v) acid.

219. The method of claim 216, wherein said alkaline pH of step f is at least pH 9.

220. A casein protein hydrolysate processed at low temperature according to the method of claim 216.

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**ABSTRACT OF THE DISCLOSURE**

Biologically active peptides that are derived from or are similar to sequences of the  $\alpha$ S1-,  $\alpha$ S2-,  $\beta$ - or  $\kappa$ -casein fractions of milk casein. These peptides are capable of immune modulation and other therapeutic activities, including but not limited to stimulating and enhancing immune response, protecting against viral infection, normalizing serum cholesterol levels, and stimulating hematopoiesis. The casein-derived peptides are non-toxic and can be used to treat and prevent immune pathologies, diabetes, hypercholesterolemia, hematological disorders and viral-related diseases.

Group >	25:1		50:1	
Ex. No v	Control	Peptides from Casein	Control	Peptides from Casein
1	16.10	43.80	27.50	62.80
2	25.70	45.40	18.20	43.40
3	0.00	3.10	0.00	35.00
4	-	-	9.00	35.00
<b>Average</b>	<b>13.93</b>	<b>30.77</b>	<b>13.68</b>	<b>44.05</b>
<b>SD</b>	<b>12.99</b>	<b>23.97</b>	<b>11.84</b>	<b>13.11</b>

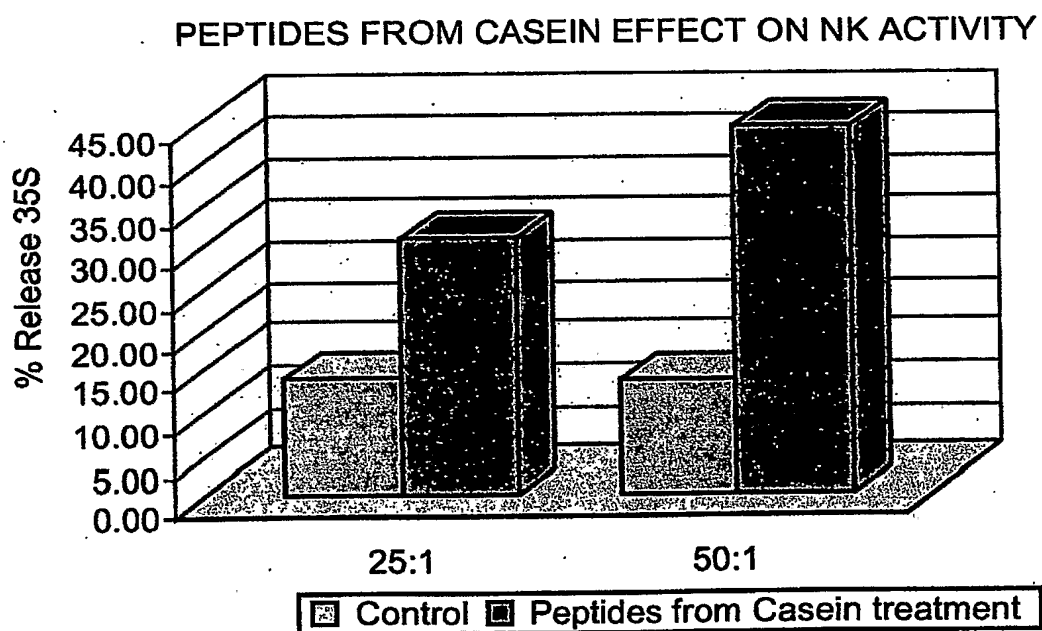


Fig. 1

Dose>	0	5	10	25	50	100	250	500
1:50	3.9	5.4	11.3	10.9	9.1	8.3	12.5	15.5
1:100	4.6	5.1	12.4	12.8	11.9	10.8	12.1	14.9

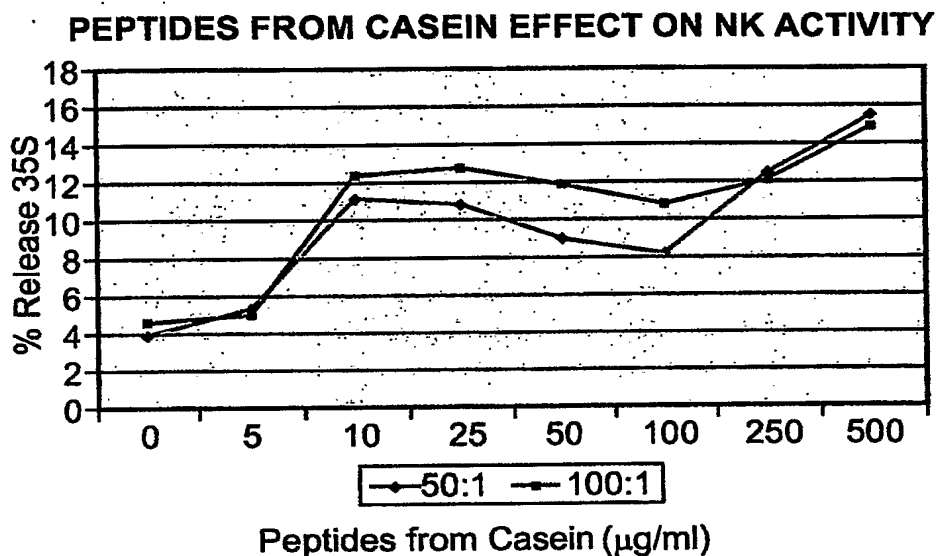


Fig. 2a

Patient	Type	0	10	25	100	250	500
1	Normal	13	15	15	12	13	15
2	NHL	10.1	13.8	14.3	-	15.8	13.7
3	NHL	3.5	10.4	8.4	10.8	-	-
4	Br.Ca	4.2	2.7	7.1	7.7	5.9	10.1
5	-	12.2	18.1	19.1	14.3	13.4	15.8
6	-	17	15	15	15	13	9

Fig. 2b

Patient	Control	Peptides from Casein
1	0.60	0.20
2	0.60	1.90
3	0.10	0.90
4	0.40	3.30
5	1.50	3.70
Mean	0.64	2.00
SD	0.52	1.50

# EFFECT OF PEPTIDES FROM CASEIN EFFECT ON NK PROLIFERATION

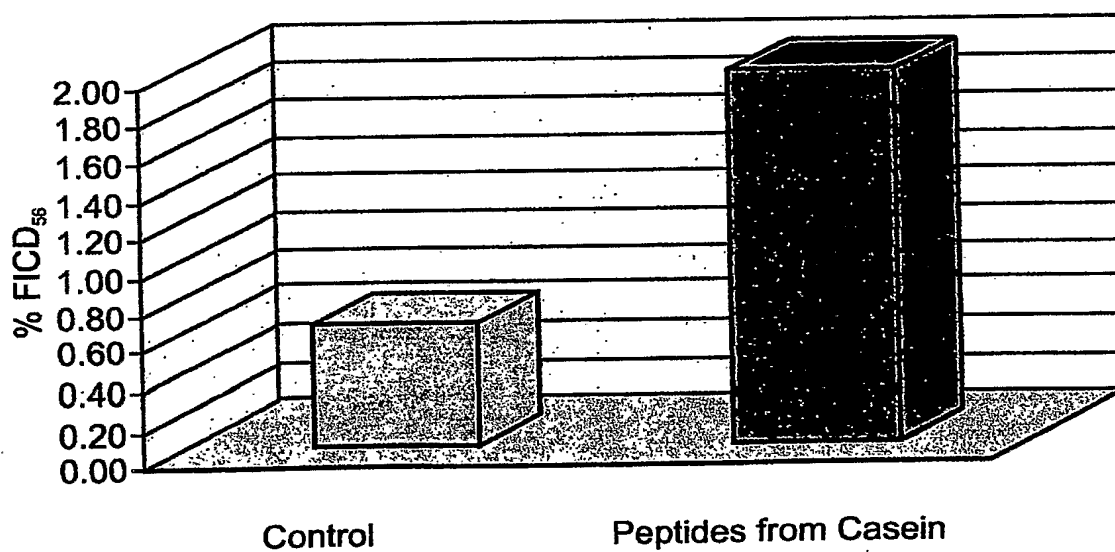


Fig. 3a

Patient	Control	Peptides from Casein
1	7.90	10.40
2	8.19	10.46
3	12.82	58.64
4	62.86	50.44
5	5.49	47.76
Mean	19.45	35.54
SD	24.41	23.27

# EFFECT OF PEPTIDES FROM CASEIN EFFECT ON T CELL PROLIFERATION

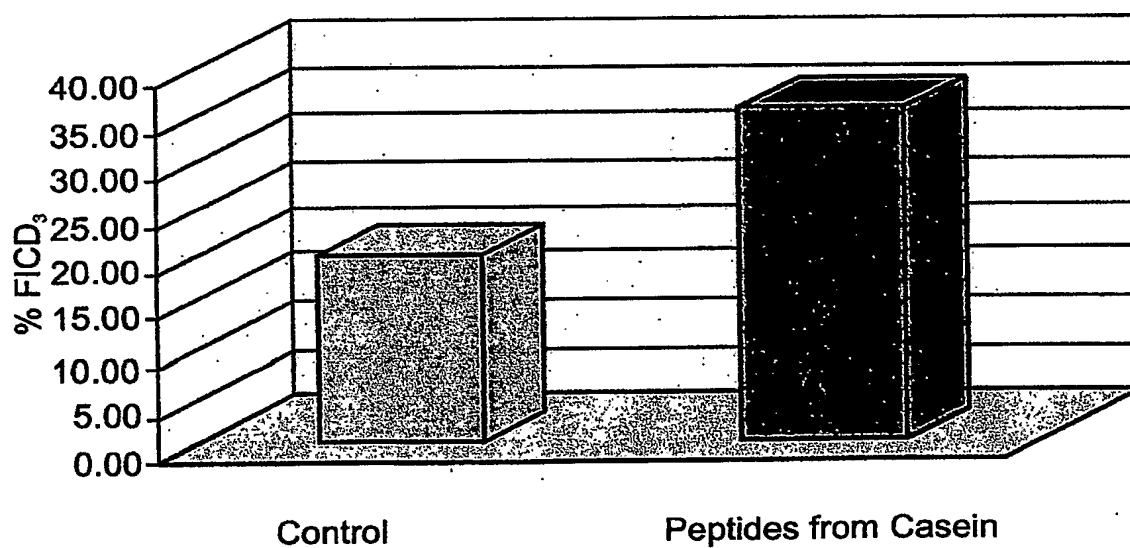


Fig. 3b

### T Cells antigens

Patient	Control	Peptides from Casein
1	8.00	25.00
2	1.1	4.3
3	0.1	0.85
4	2.77	3.89
5	1.74	4.34
6	0.84	4.53
7	0	2.55
<b>Mean</b>	2.08	6.49
<b>SD</b>	2.78	8.27

### EFFECT OF PEPTIDES FROM CASEIN ON PBSC PROLIFERATION

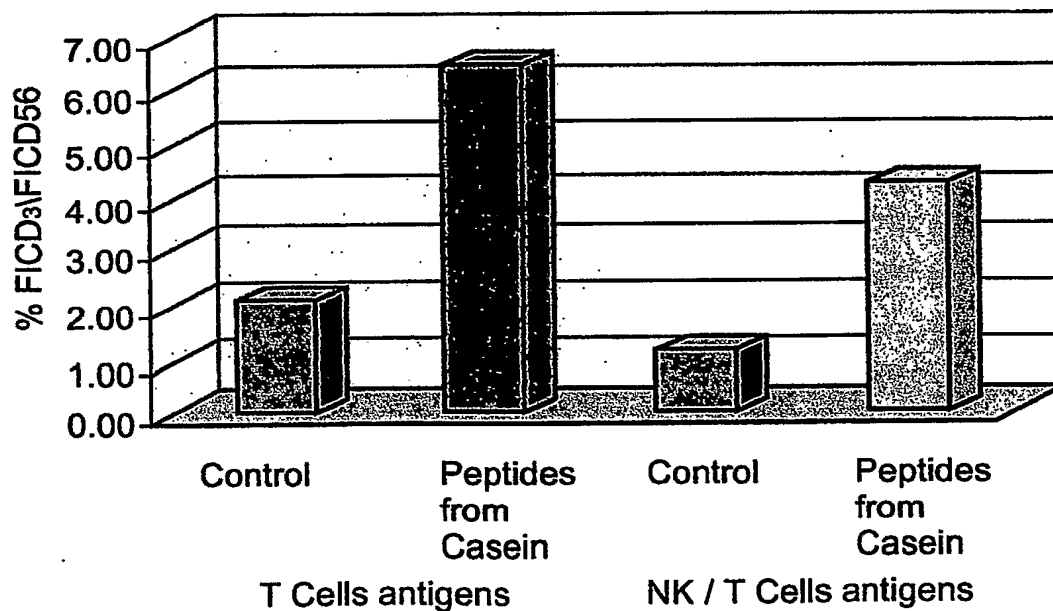


Fig. 3c

PEPTIDE 0	10 ug/ml	25 ug/ml	100 ug/ml	250 ug/ml	500 ug/ml
1a	4.3% *1880	1803 6.2%	2006 9.2%	1761 5.6%	1768 5.6%
2a	4.3% 1762	1908 7.7%	1840 6.7%	1805 6.2%	1883 7.4%
3a	4.3% 2003	1868 7.1%	1847 6.8%	1671 4.2%	1997 9.1%

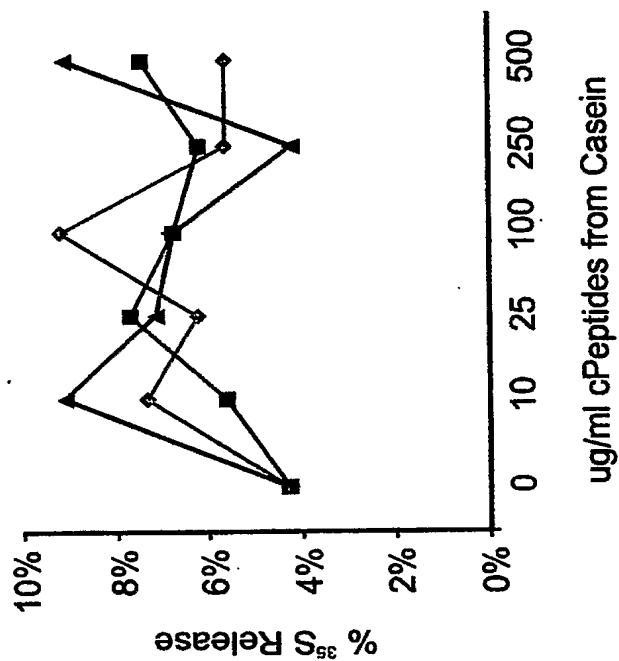
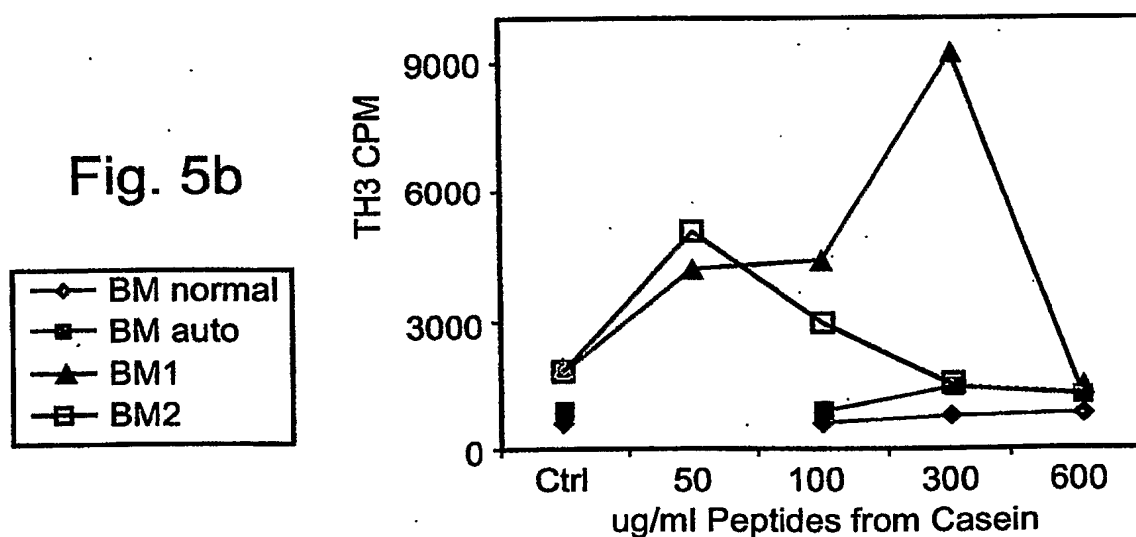
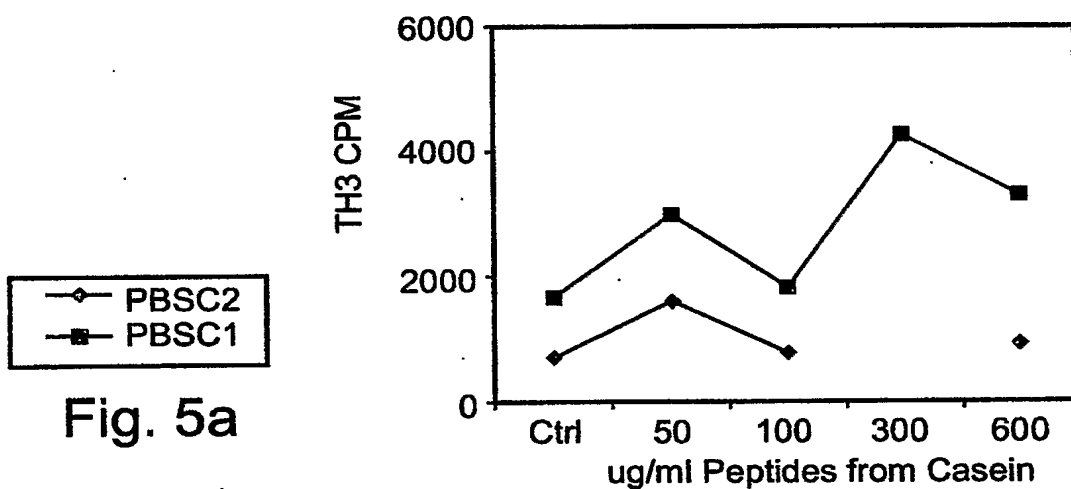


Fig. 4



Blood origin	Incubation period (days)	Control	50 ( $\mu\text{g/ml}$ )	100 ( $\mu\text{g/ml}$ )	300 ( $\mu\text{g/ml}$ )	600 ( $\mu\text{g/ml}$ )
PBSC	20	1663	3007	1800	4306	3310
PBSC	15	741	1612	784	-	920
BM Normal	21	675	-	660	834	817
BM Auto	21	945	-	916	1537	1284
BM 1	21	1829	4217	4396	9178	1446
BM 2	21	1829	5039	2939	1496	-
CB1	14	1159	1191	1694	3961	3297
CB2	14	3434	-	10882	-	13560



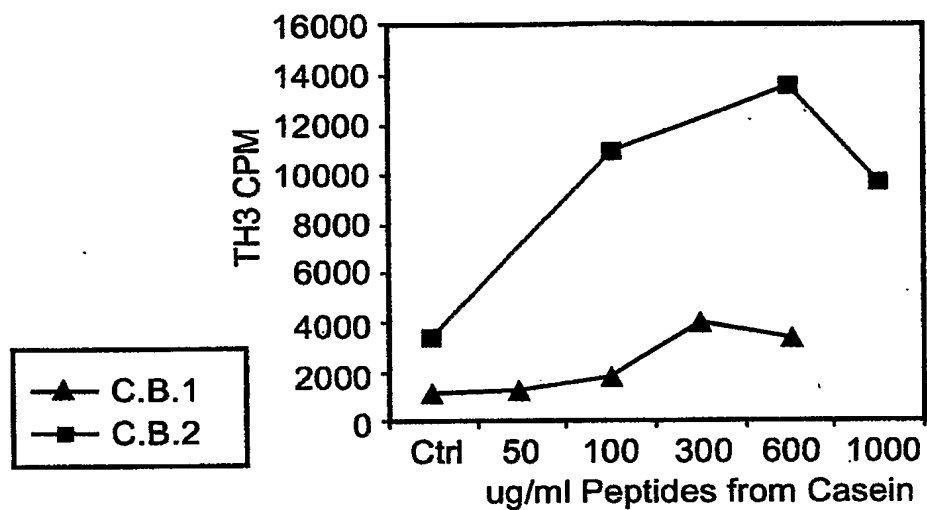


Fig. 5c

Donor	Days Of Incubation	Factors Added	Relative Cell No. X 10 <sup>4</sup> /ml μg Peptides from Casein/ml				
			0	25	100	250	500
Bone Marow	14	EPO, hIL-3, hSCF, AB serum	41	64	-	67	51
Cord Blood	13	EPO, hIL-3, hSCF, AB serum	27	158	66	50	-

Fig. 6

## Synthetic Casein-Derived Peptides

### EFFECT OF PEPTIDE LENGTH ON RELATIVE CELL DISTRIBUTION (DIFFERENTIAL COUNT) (%)

Identification	PEPTIDE'S LENGTH	CONC. ( $\mu$ g)	Mcp	PMN	EARLY MK	LATE MK	TOTAL MK	EARLY RBC	LATE RBC	TOTAL RBC	PLASMA CELLS	DENDRITIC CELLS	EOS BAS	MITOSES	TOTAL
74	2	25	17.8	2.6	3.5	3.7	7.2	15.8	20.4	36.2	8.3	23.0	2.8	4	544
1P	3	25	11.3	2.9	8.8	5.4	14.2	16.5	38.8	55.1	6.7	7.5	2.3	9	521
2P	4	25	6.1	2.3	7.4	8.1	16.5	18.4	51.8	71.2	-	-	0.6	4	700
3P	5	25	12.9	1.8	16.0	18.9	32.9	18.9	23.4	42.3	2.2	7.4	0.5	2	551
4P	6	25	22.0	3.1	21.6	24.6	46.2	5.7	11.5	17.2	0.1	4.5	4.8	4	842
5P	7	25	30.1	8.0	7.8	7.5	15.3	12.9	12.8	25.7	2.4	14.0	3.5	5	744
X	9	25	30.0	6.6	5.6	3.0	8.6	16.4	18.5	34.9	0.5	15.2	4.3	2	762
2a	11	25	8.6	1.6	14.2	28.9	43.1	13.5	28.5	40.0	3.0	3.0	0.6	12	931
2a	11	250	8.4	0.8	19.4	18.8	39.2	12.6	35.0	47.6	2.2	0.5	1.2	11	651
3a	12	25	9.5	1.8	24.1	22.5	46.6	14.0	23.4	37.4	-	3.7	1.0	16	779
D	16	25	41.0	4.5	7.0	7.8	14.6	9.6	20.2	29.8	3.4	-	6.8	7	471
D	16	250	26.6	4.8	11.9	19.4	31.3	4.2	13.1	17.3	12.3	2.4	4.5	6	620
E	17	100	15.4	5.1	12.9	14.5	27.4	20.5	23.6	44.1	4.5	1.4	2.2	7	552
E	17	1250	7.0	2.1	12.7	18.2	31.9	15.2	36.2	51.4	3.2	0.7	3.8	11	759
F	18	25	17.8	4.8	14.5	19.3	33.8	8.6	24.3	32.9	7.2	-	3.4	9	580
F	18	250	9.9	6.1	18.3	19.5	37.8	15.0	27.9	42.9	2.2	0.5	0.6	13	791
G	19	25	19.9	9.7	14.4	17.0	31.4	8.8	15.3	24.1	9.7	-	5.2	5	659
H	20	25	12.8	3.3	17.0	31.2	48.2	15.4	17.6	33.0	1.8	0.6	0.4	11	826
I	21	25	19.2	9.0	11.9	30.0	41.9	7.9	20.9	28.8	1.4	-	-	8	708
J	22	25	15.0	4.5	13.2	14.0	27.2	18.9	28.4	47.3	4.0	0.2	1.8	15	952
K	23	25	28.6	14.9	3.9	6.5	10.4	3.2	-	3.2	6.5	14.3	22.1	1	154
L	24	25	10.4	3.6	18.9	36.8	55.7	10.3	12.2	22.5	4.6	2.2	0.9	14	768
N	26	100	13.8	3.6	13.6	16.4	30.0	12.4	14.2	26.6	1.5	19.8	4.6	14	675
control (without synthetic peptides)			17.4	1.8	12.4	10.6	23.0	13.1	44.0	57.1	0.3	0.1	0.2	10	686

Fig. 7

Day After Treatment	2		4		6		9		12		15	
	Control	Peptides from Casein	Control	Peptides from Casein	Control	Peptides from Casein	Control	Peptides from Casein	Control	Peptides from Casein	Control	Peptides from Casein
1	6	9	6	32	55	55	90	205	100	280	500	800
2	10	10	18	34	40	45	135	100	160	280	440	540
3	4	6	14	40	20	85	100	130	140	220	380	800
4	6	6	8	14	35	58	130	125	280	440	600	640
5	12	6	16	18	75	60	70	155	40	340	520	600
6	8	10	18	90	25	45	85	90	320	160	380	640
Mean	7.67	7.83	13.33	38*	41.67	58*	101.67	134.17	173.33	286.67	470	670
SD	2.69	1.86	4.71	24.95	18.63	13.42	23.57	38.01	97.75	88.44	78.95	97.81

\* p<0.008

### Elevation of leukocyte reconstitution

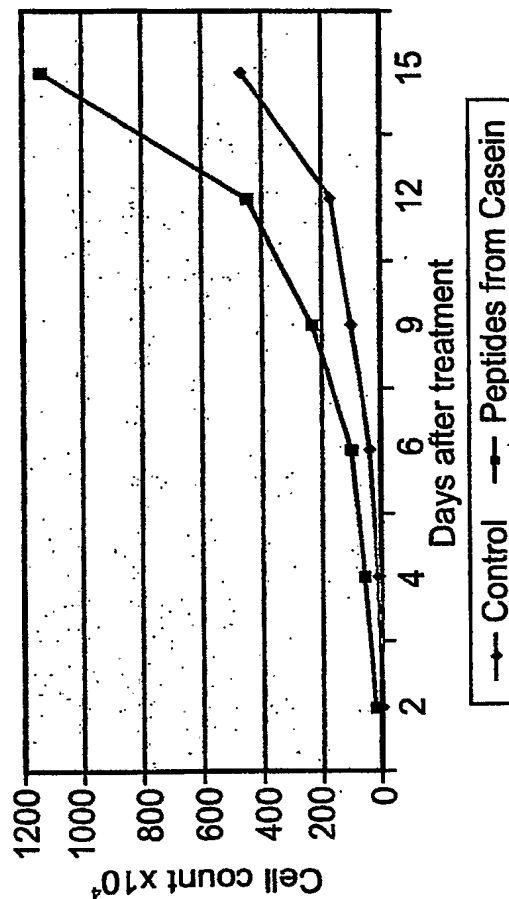


Fig. 8

Day After Treatment	11		13		15	
	Control	Peptides from Casein	Control	Peptides from Casein	Control	Peptides from Casein
1	43	50	75	103	98	110
2	48	54	71	105	99	128
3	68	68	80	110	102	111
4	64	64	104	104	96	103
5	67	67	91	101	104	133
6	63	54	90	90	97	114
7	54	45	104	107	87	104
8		63		104		116
9		61		93		115
10		57		116		112
Mean	58.14	58.3	87.86	103.3*	97.57	114.6**

\* p<0.01 \*\* p<0.0001

#### Elevation of platelets reconstitution

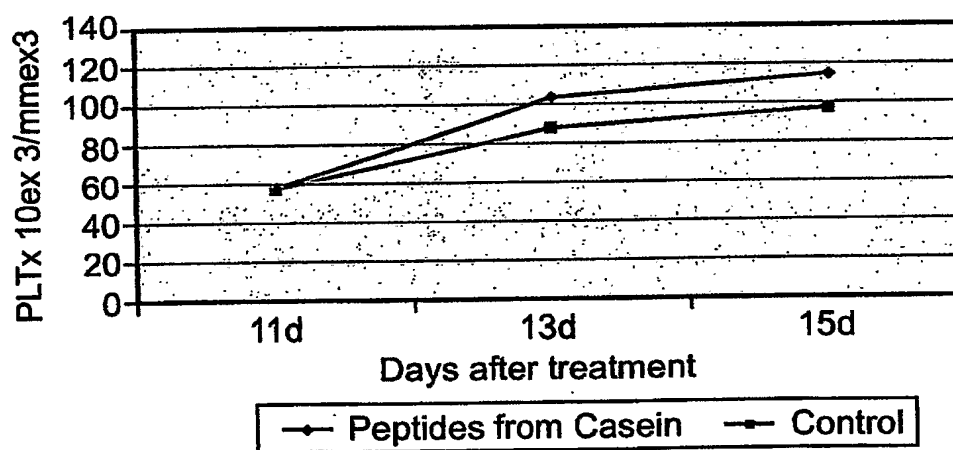


Fig. 9

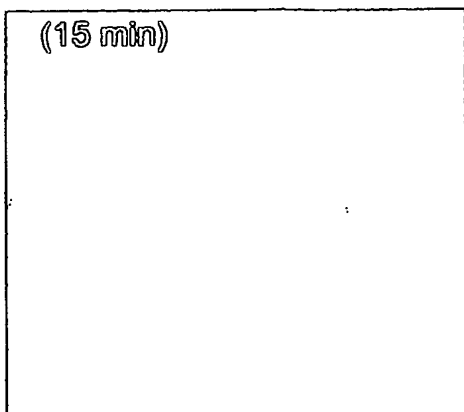


Fig. 10a

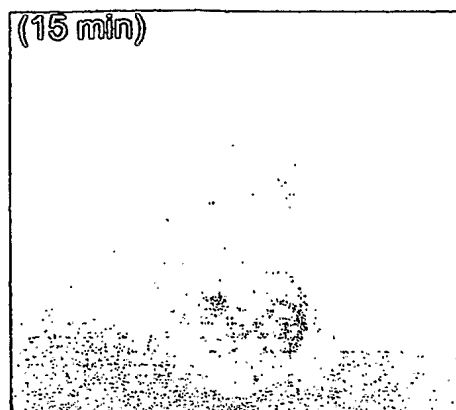


Fig. 10b

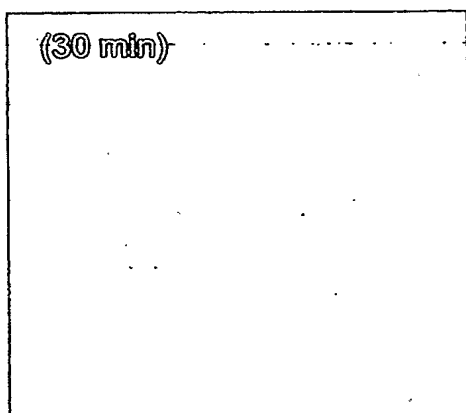


Fig. 10c

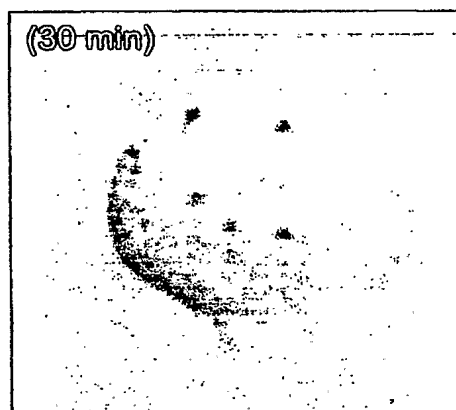


Fig. 10d

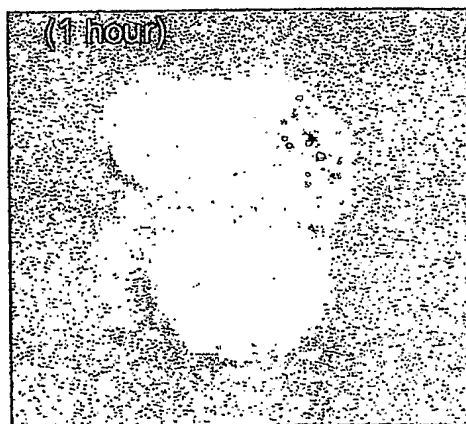


Fig. 10e

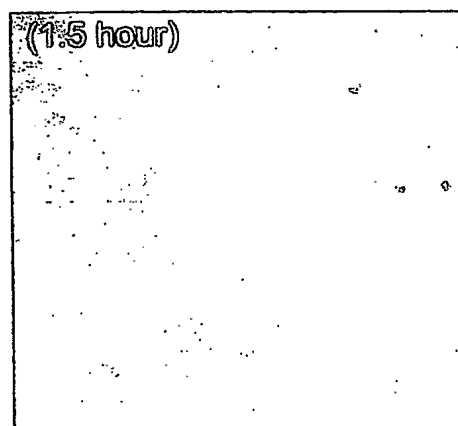


Fig. 10f

Peptides from Casein μg/ml	3 days		7 days	
	cpm Counts	Proliferation Index	cpm Counts	Proliferation Index
<b>50</b>	9268	1.18	120954	1.10
<b>100</b>	9940	1.26	112436	1.02
<b>300</b>	8425	1.07	102957	0.93
<b>600</b>	9771	1.24	101987	0.93
<b>1000</b>	8390	1.06	86649	0.79
<b>Control</b>	7862		109560	

Peptides from Casein μg/ml	10 days		14 days	
	cpm Counts	Proliferation Index	cpm Counts	Proliferation Index
<b>50</b>	17695	1.03	22272	1.36
<b>100</b>	19168	1.12	22842	1.40
<b>300</b>	21806	1.28	15318	0.93
<b>600</b>	22826	1.34	17368	1.06
<b>1000</b>	21764	1.28	10034	0.61
<b>Control</b>	17046		16313	

Fig. 11

	Peptides from Casein μg/ml	CEM cells	
		Cell No. (x10 <sup>6</sup> ) 15 days	P <sup>24</sup> Ag ng/ml
<b>3H</b>	<b>50</b>	0.29	16.39
	<b>100</b>	0.55	7.73
	<b>300</b>	0.54	1.61
	<b>600</b>	0.75	0.18
	<b>1000</b>	0.57	0.19
<b>24H</b>	<b>50</b>	0.40	0.24
	<b>100</b>	0.48	4.21
	<b>300</b>	0.56	2.94
	<b>600</b>	0.62	0.18
	<b>1000</b>	0.79	4.03
<b>48H</b>	<b>50</b>	0.37	10.05
	<b>100</b>	0.50	9.16
	<b>300</b>	0.56	3.21
	<b>600</b>	0.70	16.49
	<b>1000</b>	0.84	2.16
<b>Control</b>	<b>IF</b>	0.35	11.42
	<b>UIF</b>	0.42	0.17

Fig. 12



Peptide (3hr pre-treatment)	Conc. μg/ml	CEM cells	
		Cell No. (x10 <sup>6</sup> ) 15 days	P <sup>24</sup> Ag ng/ml
1P (SEQ ID NO 2)	100	1.29	0.17
	500	2.01	0.14
3P (SEQ ID NO 4)	10	1.17	0.26
	25	1.26	0.18
4P (SEQ ID NO 5)	25	1.26	0.42
	100	1.00	1.4
	250	1.59	0.10
Control	IF	1.06	0.52
	UIF	0.42	0.17

Fig. 13

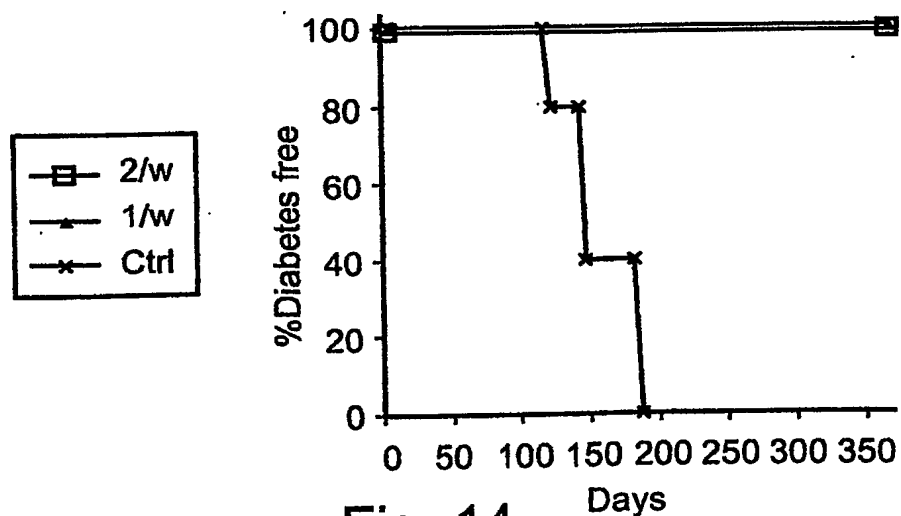


Fig. 14

Sample*	Group**	Food	TC	HDL	LDL
1	Normal	Normal	91	48	<1
2		Normal	92	56	<1
3	Control	Enriched	375	58	305
4		Enriched	411	51	348
5	B	Enriched	442	52	372
6		Enriched	445	42	386
7	C	Enriched	409	52	341
8		Enriched	411	37	361
9	2a	Enriched	279	36	229
10		Enriched	278	47	213
11	3P	Enriched	312	42	251
12		Enriched	305	43	243

\* One blood sample represents blood drawn from 2 mice.

\*\* Each group included 4 mice.

#### MEAN VALUES

		TC	HDL	LDL
1+2	Normal	91.5	52	<1
3+4	Control	393	54.5	326.5
5+6	B	449.5	47	379
7+8	C	410	44.5	351
9+10	2a	278.5	42	221
11+12	3P	308.5	42.5	247

Cholesterol, HDL & LDL in C57Bl/6 Black Mice Treated with Peptides

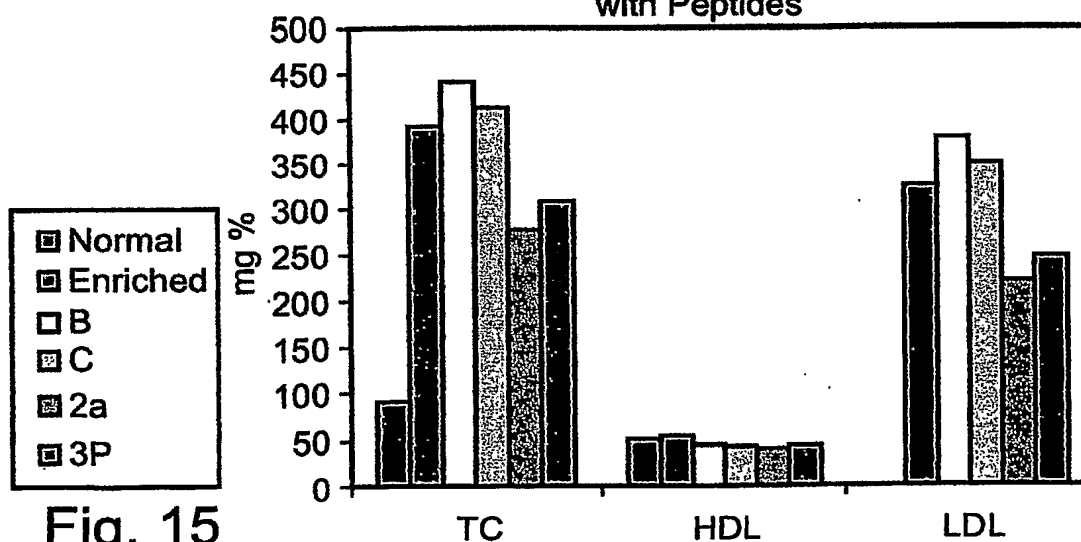


Fig. 15

Patient	WBC		PLT		RBC		HGB	
	Before	After	Before	After	Before	After	Before	After
1 G.T.	1,200 n	4,100 n+241%	17,000 n	224,000 n+1217%	3.27 n	4.05 n+23%	10.4 n	12.6 n+21%
2 E.C.	5,400 n	6,300 n+16.6%	204,000 n	259,000 n+26.9%	3.37 n	3.46 n+2.6%	10.8 n	11.0 n+1.8%
3 E.S.	3,400 n	5,100 n+50%	12,700 n	17,900 n+40%	4.49 n	4.71 n+8.4%	12.9 n	13.2 n+2.3%
4 J.R.	4,900 n	6,400 n+30%						
5 D.M.	700 n	4,600 n+557%	47,000 n	151,000 n+221%	2.88 n	3.45 n+19.7%	8.6 n	10.5 n+22%

WBC - White blood cells  
 PLT - Platelets  
 RBC - Red blood cells  
 HGB - Hemoglobin

Fig. 16

X	Y
0	11
1	10
3	10
5	32.5
7	15
8	27.5
12	40
14.25	28
17	35
21	45
26.35	70.3
31.7	74
40	100.7

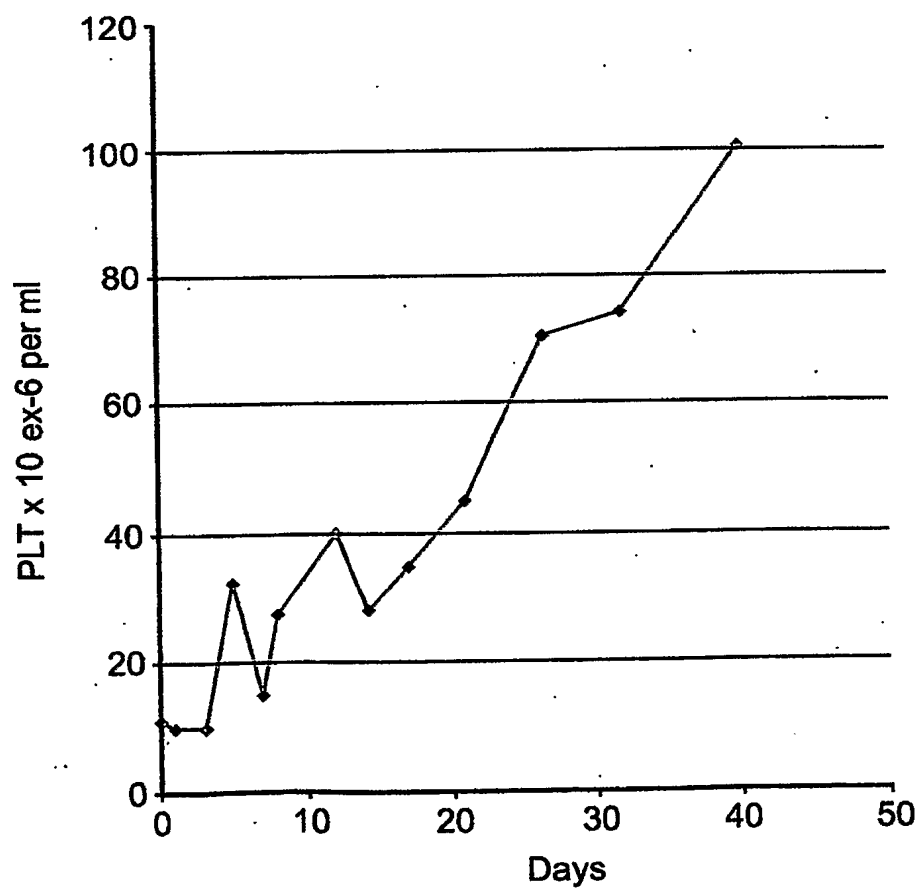


Fig. 17

<u>X</u>	<u>Y</u>
0	23
1	18.5
2	25
3	16
4	20.8
6	20.8
7	20
8	23.5
9	26
10	19.5
11	23
13	18.5
14	18.5
15	20
17.2	22
20.3	30
24	44
29	75.6
36.5	86.4
41	139.5

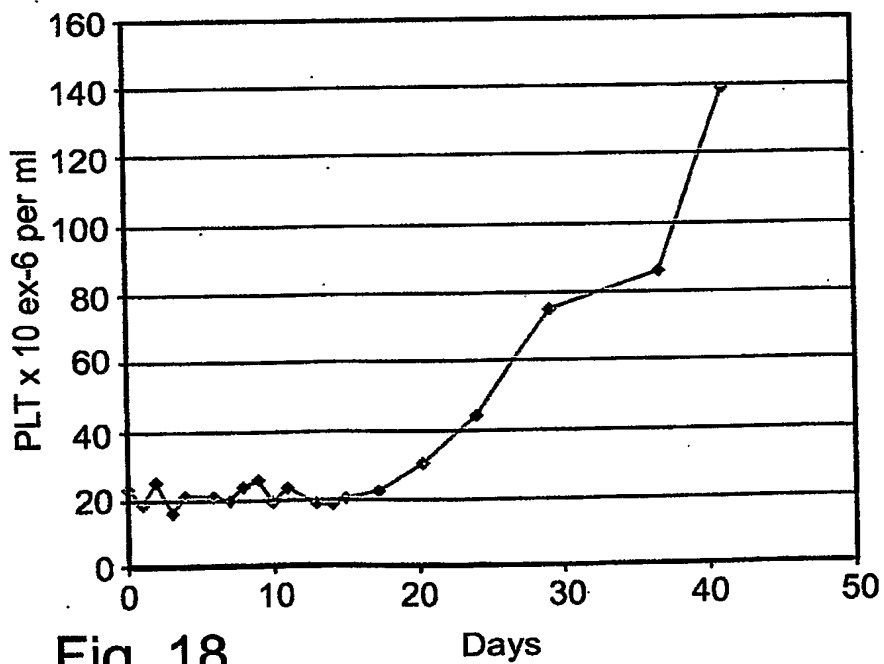


Fig. 18

**Myeloid Colonies /  $1 \times 10^5$  MNC plated (CFU-GM)**  
**CFU-GM**

Factor added	Colonies per $10^5$ MNC Plated
Control + IL-3	52
G-CSF+ IL-3	61
30-4 + IL-3	58
J + IL-3	52
G-CSF+ 30-4 + IL-3	72
G-CSF+ J + IL-3	76

**Fig. 19**

**Myeloid Colonies /  $1 \times 10^5$  MNC plated (CFU-GM)**  
**CFU-GM**

Factor added	Conc.	Colonies per $10^5$ MNC Plated	Enhancement of Response to GCSF
G-CSF	75 units/ml	50	0
J + G-CSF	100 $\mu$ g/ml	77	1.54
	300 $\mu$ g/ml	60	1.2
$\beta$ + G-CSF	100 $\mu$ g/ml	58	1.16
	300 $\mu$ g/ml	65	1.3

**Fig. 20**

**Percent Megakaryocytes of Total Cells Counted**

Factor Added	Conc.	Early MK	Late MK	Total MK
Control		4.4	13.6	18.0
Synthetic Kappa (106-127)(SEQ ID NO: 30)	25 $\mu$ g	6.8	15.0	21.8
Synthetic Beta (193-208)(SEQ ID NO: 28)	25 $\mu$ g	7.5	16.4	23.9
Synthetic Alpha-S1 (1-22)(SEQ ID NO:21)	25 $\mu$ g	12.7	15.5	28.2

**Fig. 21**

**Number of Colonies from Murine Bone Marrow Progenitor Cells  
(CFU-GEMM)**

Factor Added	Days of Incubation	Conc. µg/ml	
		0	25
β (SEQ ID NO: 28)	8	17	38
κ (SEQ ID NO: 30)	8	17	36
β + κ	8	17	62

**Fig. 22**

**Platelet reconstitution**

Factor added	Platelet count ( $\times 10^3$ ) per ml at 10 days
Control	332
J (SEQ ID NO: 21) 1mg	445
Control	338
β (SEQ ID NO: 28) 1mg	447
Control	370
κ (SEQ ID NO: 30) 1mg	468

**Fig. 23**

**Leukocyte Proliferation (Mean WBC counts)**

Factor Added	5 Days	7 Days	10 Days
α-S1(1-23)	$5.25 \times 10^4$	$52.5 \times 10^4$	$1.80 \times 10^6$
κ-casein (106-169)	$7.20 \times 10^4$	$79.0 \times 10^4$	$1.76 \times 10^6$
β-casein(Synthetic) (SEQ ID NO: 28)	$17.4 \times 10^4$	$56.0 \times 10^4$	$1.90 \times 10^6$
α-S1casein(1-22)(Synthetic) (SEQ ID NO: 21)	$7.80 \times 10^4$	$72.0 \times 10^4$	$1.70 \times 10^6$
Control	$4.80 \times 10^4$	$39.0 \times 10^4$	$1.56 \times 10^6$

**Fig. 24**

**Leukocyte Proliferation (Mean WBC counts)**

Factor added	WBC ( $\times 10^3$ per mm <sup>3</sup> ) at		
	day 4	day 10	day 12
J (αS1 1-22) (SEQ ID NO: 21)	2.3	35.8	35.2
β-casein (193-208) (SEQ ID NO: 28)	4.0	28.0	32.8
J+ β	3.0	31.0	41.0
Saline	2.2	25.2	36.8

**Fig. 25**

### Chimeric Peptides of $\alpha$ S1- and $\beta$ -casein

$\alpha$ S1-peptide	SEQ ID NO:	$\beta$ - peptide YQ	SEQ ID NO:	$\beta$ - peptide YQE
RP	34	RPYQ	35	RPYQE
RPK	36	RPKYQ	37	RPKYQE
RPKH	38	RPKHYQ	39	RPKHYQE
RPKHP	40	RPKHPYQ	41	RPKHPYQE
RPKHPI	42	RPKHPIYQ	43	RPKHPIYQE
RPKHPIK	44	RPKHPIKYQ	45	RPKHPIKYQE
RPKHPIKH	46	RPKHPIKH YQ	47	RPKHPIKH YQE
RPKHPIKHQ	48	RPKHPIKHQYQ	49	RPKHPIKHQYQE
RPKHPIKHQG	50	RPKHPIKHQGYQ	51	RPKHPIKHQGYQE
RPKHPIKHQGL	52	RPKHPIKHQGLYQ	53	RPKHPIKHQGLYQE
RPKHPIKHQGLP	54	RPKHPIKHQGLPYQ	55	RPKHPIKHQGLPYQE
RPKHPIKHQGLPQ	56	RPKHPIKHQGLPQYQ	57	RPKHPIKHQGLPQYQE
RPKHPIKHQGLPQE	58	RPKHPIKHQGLPQEYQ	59	RPKHPIKHQGLPQEYQE
RPKHPIKHQGLPQEV	60	RPKHPIKHQGLPQEVYQ	61	RPKHPIKHQGLPQEVYQE
RPKHPIKHQGLPQEV L	62	RPKHPIKHQGLPQEVLYQ	63	RPKHPIKHQGLPQEVLYQ E
RPKHPIKHQGLPQEV L N	64	RPKHPIKHQGLPQEV LNYQ	65	RPKHPIKHQGLPQEV LNY QE
RPKHPIKHQGLPQEV L NE	66	RPKHPIKHQGLPQEV LNEYQ	67	RPKHPIKHQGLPQEV LNEY QE

Fig. 26a  
 Fig. 26b  
 Fig. 26c  
 Fig. 26d  
 Fig. 26e  
 Fig. 26f  
 Fig. 26g  
 Fig. 26h  
 Fig. 26i  
 Fig. 26

Fig. 26a



RPKHPIKHQGLPQEV NEN	68	RPKHPIKHQGLPQEVNENYQ	69	RPKHPIKHQGLPQEVNEN YQE
RPKHPIKHQGLPQEV NENL	70	RPKHPIKHQGLPQEVNENLY Q	71	RPKHPIKHQGLPQEVNEN LYQE
RPKHPIKHQGLPQEV NENLL	72	RPKHPIKHQGLPQEVNENLL YQ	73	RPKHPIKHQGLPQEVNEN LLYQE
RPKHPIKHQGLPQEV NENLLR	74	RPKHPIKHQGLPQEVNENLL RYQ	75	RPKHPIKHQGLPQEVNEN LLRYQE
RPKHPIKHQGLPQEV NENLLRF	76	RPKHPIKHQGLPQEVNENLL RFYQ	77	RPKHPIKHQGLPQEVNEN LLRFYQE
RPKHPIKHQGLPQEV NENLLRFF	78	RPKHPIKHQGLPQEVNENLL RFFYQ	79	RPKHPIKHQGLPQEVNEN LLRFFYQE
RPKHPIKHQGLPQEV NENLLRFFV	80	RPKHPIKHQGLPQEVNENLL RFFVYQ	81	RPKHPIKHQGLPQEVNEN LLRFFVYQE
RPKHPIKHQGLPQEV NENLLRFFVA	82	RPKHPIKHQGLPQEVNENLL RFFVAYQ	83	RPKHPIKHQGLPQEVNEN LLRFFVAYQE
	SEQ ID NO:	YQEP	SEQ ID NO:	YQEPV
RP	84	RPYQEP	85	RPYQEPV
RPK	86	RPKYQEP	87	RPKYQEPV
RPKH	88	RPKHYQEP	89	RPKHYQEPV
RPKHIP	90	RPKHPIYQEP	91	RPKHPIYQEPV
RPKHPI	92	RPKHPIYQEP	93	RPKHPIYQEPV
RPKHPIK	94	RPKHPIKYQEP	95	RPKHPIKYQEPV
RPKHPIKH	96	RPKHPIKHYQEP	97	RPKHPIKHYQEPV
RPKHPIKHQ	98	RPKHPIKHQYQEP	99	RPKHPIKHQYQEPV
RPKHPIKHQG	100	RPKHPIKHQGYQEP	101	RPKHPIKHQGYQEPV
RPKHPIKHQGL	102	RPKHPIKHQGLYQEP	103	RPKHPIKHQGLYQEPV
RPKHPIKHQGLP	104	RPKHPIKHQGLPYQEP	105	RPKHPIKHQGLPYQEPV
RPKHPIKHQGLPQ	106	RPKHPIKHQGLPQYQEP	107	RPKHPIKHQGLPQYQEPV
RPKHPIKHQGLPQE	108	RPKHPIKHQGLPQEYQEP	109	RPKHPIKHQGLPQEYQEP V
RPKHPIKHQGLPQEV	110	RPKHPIKHQGLPQEVYQEP	111	RPKHPIKHQGLPQEVYQEP V
RPKHPIKHQGLPQEV N	112	RPKHPIKHQGLPQEVLYQEP	113	RPKHPIKHQGLPQEVLYQ EPV
RPKHPIKHQGLPQEV NE	114	RPKHPIKHQGLPQEVNLYQEP	115	RPKHPIKHQGLPQEVNLY QEPV
RPKHPIKHQGLPQEV NE	116	RPKHPIKHQGLPQEVNLYQEP P	117	RPKHPIKHQGLPQEVNEN LYQEPV
RPKHPIKHQGLPQEV NEN	118	RPKHPIKHQGLPQEVNENYQ EP	119	RPKHPIKHQGLPQEVNEN LYQEPV

Fig. 26b

RPKHPIKHQGLPQEV NENL	120	RPKHPIKHQGLPQEVNENLY QEP	121	RPKHPIKHQGLPQEVNENLY QEPV
RPKHPIKHQGLPQEV NENLL	122	RPKHPIKHQGLPQEVNENLL YQEP	123	RPKHPIKHQGLPQEVNENLL YQEPV
RPKHPIKHQGLPQEV NENLLR	124	RPKHPIKHQGLPQEVNENLL RYQEP	125	RPKHPIKHQGLPQEVNENLL RYQEPV
RPKHPIKHQGLPQEV NENLLRF	126	RPKHPIKHQGLPQEVNENLL RFYQEP	127	RPKHPIKHQGLPQEVNENLL RFYQEPV
RPKHPIKHQGLPQEV NENLLRFF	128	RPKHPIKHQGLPQEVNENLL RFFYQEP	129	RPKHPIKHQGLPQEVNENLL RFFYQEPV
RPKHPIKHQGLPQEV NENLLRFFV	130	RPKHPIKHQGLPQEVNENLL RFFVYQEP	131	RPKHPIKHQGLPQEVNENLL RFFVYQEPV
RPKHPIKHQGLPQEV NENLLRFFVA	132	RPKHPIKHQGLPQEVNENLL RFFVAYQEP	133	RPKHPIKHQGLPQEVNENLL RFFVAYQEPV
	SEQ ID NO:	YQEPVL	SEQ ID NO:	YQEPVLG
RP	134	RPYQEPVL	135	RPYQEPVLG
RPK	136	RPKYQEPVL	137	RPKYQEPVLG
RPKH	138	RPKHYPQEPVL	139	RPKHYPQEPVLG
RPKHP	140	RPKHYPQEPVL	141	RPKHYPQEPVLG
RPKHPI	142	RPKHPIYPQEPVL	143	RPKHPIYPQEPVLG
RPKHPIK	144	RPKHPIKYQEPVL	145	RPKHPIKYQEPVLG
RPKHPIKH	146	RPKHPIKYPQEPVL	147	RPKHPIKYPQEPVLG
RPKHPIKHQ	148	RPKHPIKHYPQEPVL	149	RPKHPIKHYPQEPVLG
RPKHPIKHQG	150	RPKHPIKHQYPQEPVL	151	RPKHPIKHQYPQEPVLG
RPKHPIKHQGL	152	RPKHPIKHQGLYPQEPVL	153	RPKHPIKHQGLYPQEPVLG
RPKHPIKHQGLP	154	RPKHPIKHQGLPYQEPVL	155	RPKHPIKHQGLPYQEPVL G
RPKHPIKHQGLPQ	156	RPKHPIKHQGLPQYPQEPVL	157	RPKHPIKHQGLPQYPQEPV LG
RPKHPIKHQGLPQE	158	RPKHPIKHQGLPQEYPQEPVL	159	RPKHPIKHQGLPQEYPQEP VLG
RPKHPIKHQGLPQEV	160	RPKHPIKHQGLPQEVYPQEPVL	161	RPKHPIKHQGLPQEVYPQEP VLG
RPKHPIKHQGLPQEV L	162	RPKHPIKHQGLPQEVLYQEPV L	163	RPKHPIKHQGLPQEVLYQ EPVLG
RPKHPIKHQGLPQEV N	164	RPKHPIKHQGLPQEVLYQEP VL	165	RPKHPIKHQGLPQEVLYQ EPVLG
RPKHPIKHQGLPQEV NE	166	RPKHPIKHQGLPQEVLYQEP PVL	167	RPKHPIKHQGLPQEVLYQ EPVLG
RPKHPIKHQGLPQEV NEN	168	RPKHPIKHQGLPQEVLYQEP EPVL	169	RPKHPIKHQGLPQEVLYQ EPVLG
RPKHPIKHQGLPQEV NENL	170	RPKHPIKHQGLPQEVLYQEP QEPVL	171	RPKHPIKHQGLPQEVLYQ EPVLG

Fig. 26c

RPKHPIKHQGLPQEV NENLL	172	RPKHPIKHQGLPQEVNENLL YQEPVL	173	RPKHPIKHQGLPQEVNEN LLYQEPVLG
RPKHPIKHQGLPQEV NENLLR	174	RPKHPIKHQGLPQEVNENLL RYQEPVL	175	RPKHPIKHQGLPQEVNEN LLRYQEPVLG
RPKHPIKHQGLPQEV NENLLRF	176	RPKHPIKHQGLPQEVNENLL RFYQEPVL	177	RPKHPIKHQGLPQEVNEN LLRFYQEPVLG
RPKHPIKHQGLPQEV NENLLRFF	178	RPKHPIKHQGLPQEVNENLL RFFYQEPVL	179	RPKHPIKHQGLPQEVNEN LLRFFYQEPVLG
RPKHPIKHQGLPQEV NENLLRFFV	180	RPKHPIKHQGLPQEVNENLL RFFVYQEPVL	181	RPKHPIKHQGLPQEVNEN LLRFFVYQEPVLG
RPKHPIKHQGLPQEV NENLLRFFVA	182	RPKHPIKHQGLPQEVNENLL RFFVAYQEPVL	183	RPKHPIKHQGLPQEVNEN LLRFFVAYQEPVLG
	SEQ ID NO:	YQEPVLGP	SEQ ID NO:	YQEPVLGPV
RP	184	RPYQEPVLGP	185	RPYQEPVLGPV
RPK	186	RPKYQEPVLGP	187	RPKYQEPVLGPV
RPKH	188	RPKHYPQEPVLGP	189	RPKHYPQEPVLGPV
RPKHP	190	RPKHYPQEPVLGP	191	RPKHYPQEPVLGPV
RPKHPI	192	RPKHPIYQEPVLGP	193	RPKHPIYQEPVLGPV
RPKHPIK	194	RPKHPIKYQEPVLGP	195	RPKHPIKYQEPVLGPV
RPKHPIKH	196	RPKHPIKHYQEPVLGP	197	RPKHPIKHYQEPVLGPV
RPKHPIKHQ	198	RPKHPIKHQYQEPVLGP	199	RPKHPIKHQYQEPVLGPV
RPKHPIKHQG	200	RPKHPIKHQGYQEPVLGP	201	RPKHPIKHQGYQEPVLGP V
RPKHPIKHQGL	202	RPKHPIKHQGLYQEPVLGP	203	RPKHPIKHQGLYQEPVLG PV
RPKHPIKHQGLP	204	RPKHPIKHQGLPYQEPVLGP	205	RPKHPIKHQGLPYQEPVL GPV
RPKHPIKHQGLPQ	206	RPKHPIKHQGLPQYQEPVLGP	207	RPKHPIKHQGLPQYQEPV LGPV
RPKHPIKHQGLPQE	208	RPKHPIKHQGLPQEQYQEPVLG P	209	RPKHPIKHQGLPQEQYQEP VLGPV
RPKHPIKHQGLPQEV	210	RPKHPIKHQGLPQEVYQEPVL GP	211	RPKHPIKHQGLPQEVYQE PVLGPV
RPKHPIKHQGLPQEV L	212	RPKHPIKHQGLPQEVLYQEPV LGP	213	RPKHPIKHQGLPQEVLYQ EPVLGPV
RPKHPIKHQGLPQEV N	214	RPKHPIKHQGLPQEVNLYQEP VLGP	215	RPKHPIKHQGLPQEVNLY QEPVLGPV
RPKHPIKHQGLPQEV NE	216	RPKHPIKHQGLPQEVNEYQE PVLGP	217	RPKHPIKHQGLPQEVNEN YQEPVLGPV
RPKHPIKHQGLPQEV NEN	218	RPKHPIKHQGLPQEVNENYQ EPVLGP	219	RPKHPIKHQGLPQEVNEN YQEPVLGPV
RPKHPIKHQGLPQEV NENL	220	RPKHPIKHQGLPQEVNENLY QEPVLGP	221	RPKHPIKHQGLPQEVNEN LYQEPVLGPV
RPKHPIKHQGLPQEV NENLL	222	RPKHPIKHQGLPQEVNENLL YQEPVLGP	223	RPKHPIKHQGLPQEVNEN LLYQEPVLGPV

Fig. 26d

RPKHPIKHQGLPQEV NENLLR	224	RPKHPIKHQGLPQEV NENLL RYQEPVLGP	225	RPKHPIKHQGLPQEV NENLL NLLRYQEPVLGPV
RPKHPIKHQGLPQEV NENLLRF	226	RPKHPIKHQGLPQEV NENLL RFYQEPVLGP	227	RPKHPIKHQGLPQEV NENLL NLLRFYQEPVLGPV
RPKHPIKHQGLPQEV NENLLRFF	228	RPKHPIKHQGLPQEV NENLL RFFYQEPVLGP	229	RPKHPIKHQGLPQEV NENLL NLLRFFYQEPVLGPV
RPKHPIKHQGLPQEV NENLLRFFV	230	RPKHPIKHQGLPQEV NENLL RFFVYQEPVLGP	231	RPKHPIKHQGLPQEV NENLL NLLRFFVYQEPVLGPV
RPKHPIKHQGLPQEV NENLLRFFVA	232	RPKHPIKHQGLPQEV NENLL RFFVAYQEPVLGP	233	RPKHPIKHQGLPQEV NENLL NLLRFFVAYQEPVLGPV
	SEQ ID NO:	YQEPVLGPVR	SEQ ID NO:	YQEPVLGPVRG
RP	234	RPYQEPVLGPVR	235	RPYQEPVLGPVRG
RPK	236	RPKYQEPVLGPVR	237	RPKYQEPVLGPVRG
RPKH	238	RPKHQYQEPVLGPVR	239	RPKHQYQEPVLGPVRG
RPKHP	240	RPKHQYQEPVLGPVR	241	RPKHQYQEPVLGPVRG
RPKHPI	242	RPKHPIYQEPVLGPVR	243	RPKHPIYQEPVLGPVRG
RPKHPIK	244	RPKHPIKYQEPVLGPVR	245	RPKHPIKYQEPVLGPVRG
RPKHPIKH	246	RPKHPIKHQYQEPVLGPVR	247	RPKHPIKHQYQEPVLGPVR G
RPKHPIKHQ	248	RPKHPIKHQYQEPVLGPVR	249	RPKHPIKHQYQEPVLGPVR G
RPKHPIKHQG	250	RPKHPIKHQGYQEPVLGPVR	251	RPKHPIKHQGYQEPVLGPVR G
RPKHPIKHQGL	252	RPKHPIKHQGLYQEPVLGPVR	253	RPKHPIKHQGLYQEPVLGPVR G
RPKHPIKHQGLP	254	RPKHPIKHQGLPYQEPVLGPVR	255	RPKHPIKHQGLPYQEPVLGPVR G
RPKHPIKHQGLPQ	256	RPKHPIKHQGLPQYQEPVLGPVR	257	RPKHPIKHQGLPQYQEPVLGPVR G
RPKHPIKHQGLPQE	258	RPKHPIKHQGLPQEYQEPVLGPVR	259	RPKHPIKHQGLPQEYQEPVLGPVR G
RPKHPIKHQGLPQEV	260	RPKHPIKHQGLPQEVYQEPVLGPVR	261	RPKHPIKHQGLPQEVYQEPVLGPVR G
RPKHPIKHQGLPQEV L	262	RPKHPIKHQGLPQEVLYQEPVLGPVR	263	RPKHPIKHQGLPQEVLYQEPVLGPVR G
RPKHPIKHQGLPQEV N	264	RPKHPIKHQGLPQEVLYQEPVLGPVR	265	RPKHPIKHQGLPQEVLYQEPVLGPVR G
RPKHPIKHQGLPQEV NE	266	RPKHPIKHQGLPQEVLYQEPVLGPVR	267	RPKHPIKHQGLPQEVLYQEPVLGPVR G
RPKHPIKHQGLPQEV NEN	268	RPKHPIKHQGLPQEVLYQEPVLGPVR	269	RPKHPIKHQGLPQEVLYQEPVLGPVR G
RPKHPIKHQGLPQEV NENL	270	RPKHPIKHQGLPQEVLYQEPVLGPVR	271	RPKHPIKHQGLPQEVLYQEPVLGPVR G
RPKHPIKHQGLPQEV NENLL	272	RPKHPIKHQGLPQEVLYQEPVLGPVR	273	RPKHPIKHQGLPQEVLYQEPVLGPVR G
RPKHPIKHQGLPQEV NENLLR	274	RPKHPIKHQGLPQEVLYQEPVLGPVR	275	RPKHPIKHQGLPQEVLYQEPVLGPVR G

Fig. 26e

RPKHPIKHQGLPQEV NENLLRF	276	RPKHPIKHQGLPQEVNENLL RFYQEPVLPVR	277	RPKHPIKHQGLPQEVNEN LLRFYQEPVLPVRG
RPKHPIKHQGLPQEV NENLLRFF	278	RPKHPIKHQGLPQEVNENLL RFFYQEPVLPVR	279	RPKHPIKHQGLPQEVNEN LLRFFYQEPVLPVRG
RPKHPIKHQGLPQEV NENLLRFFV	280	RPKHPIKHQGLPQEVNENLL RFFVYQEPVLPVR	281	RPKHPIKHQGLPQEVNEN LLRFFVYQEPVLPVRG
RPKHPIKHQGLPQEV NENLLRFFVA	282	RPKHPIKHQGLPQEVNENLL RFFVAYQEPVLPVR	283	RPKHPIKHQGLPQEVNEN LLRFFVAYQEPVLPVR G
	SEQ ID NO:		SEQ ID NO:	
		VQEPVLPVRGP		VQEPVLPVRGPF
RP	284	RPYQEPVLPVRGP	285	RPYQEPVLPVRGPF
RPK	286	RPKYQEPVLPVRGP	287	RPKYQEPVLPVRGPF
RPKH	288	RPKHQEPVLPVRGP	289	RPKHQEPVLPVRGPF
RPKHP	290	RPKHYPQEPVLPVRGP	291	RPKHYPQEPVLPVRGP F
RPKHPI	292	RPKHPIYQEPVLPVRGP	293	RPKHPIYQEPVLPVRGP F
RPKHPIK	294	RPKHPIKYQEPVLPVRGP	295	RPKHPIKYQEPVLPVRG PF
RPKHPIKH	296	RPKHPIKHQEPVLPVRGP	297	RPKHPIKHQEPVLPVR GPF
RPKHPIKHQ	298	RPKHPIKHQYQEPVLPVRGP	299	RPKHPIKHQYQEPVLPV RGPF
RPKHPIKHQG	300	RPKHPIKHQGYQEPVLPVRG P	301	RPKHPIKHQGYQEPVLP VRGPF
RPKHPIKHQGL	302	RPKHPIKHQGLYQEPVLPVR GP	303	RPKHPIKHQGLYQEPVLG PVRGPF
RPKHPIKHQGLP	304	RPKHPIKHQGLPYQEPVLPV RGP	305	RPKHPIKHQGLPYQEPVL GPVRGPF
RPKHPIKHQGLPQ	306	RPKHPIKHQGLPQYQEPVLP VRGP	307	RPKHPIKHQGLPQYQEPV LGPVRGPF
RPKHPIKHQGLPQE	308	RPKHPIKHQGLPQEYQEPVLG PVRGP	309	RPKHPIKHQGLPQEYQEP VLGPVRGPF
RPKHPIKHQGLPQEV	310	RPKHPIKHQGLPQEVYQEPVL GPVRGP	311	RPKHPIKHQGLPQEVYQE PVLGPVRGPF
RPKHPIKHQGLPQEV L	312	RPKHPIKHQGLPQEVLYQEPV LGPVRGP	313	RPKHPIKHQGLPQEVLYQ EPVLPVRGPF
RPKHPIKHQGLPQEV N	314	RPKHPIKHQGLPQEVNLYQEP VLGPVRGP	315	RPKHPIKHQGLPQEVNLY QEPVLPVRGPF
RPKHPIKHQGLPQEV NE	316	RPKHPIKHQGLPQEVNLYQE PVLGPVRGP	317	RPKHPIKHQGLPQEVNLY QEPVLPVRGPF
RPKHPIKHQGLPQEV NEN	318	RPKHPIKHQGLPQEVNENLYQ EPVLPVRGP	319	RPKHPIKHQGLPQEVNEN LYQEPVLPVRGPF
RPKHPIKHQGLPQEV NENL	320	RPKHPIKHQGLPQEVNENLY QEPVLPVRGP	321	RPKHPIKHQGLPQEVNEN LYQEPVLPVRGPF
RPKHPIKHQGLPQEV NENLL	322	RPKHPIKHQGLPQEVNENLL YQEPVLPVRGP	323	RPKHPIKHQGLPQEVNEN LLYQEPVLPVRGPF
RPKHPIKHQGLPQEV NENLLR	324	RPKHPIKHQGLPQEVNENLL RYQEPVLPVRGP	325	RPKHPIKHQGLPQEVNEN LLRYQEPVLPVRGPF

Fig. 26f

RPKHPIKHQGLPQEV NENLLRF	326	RPKHPIKHQGLPQEVNENLL RFYQEPVLPVRGP	327	RPKHPIKHQGLPQEVNENLL NLLRFYQEPVLPVRGP
RPKHPIKHQGLPQEV NENLLRFF	328	RPKHPIKHQGLPQEVNENLL RFFYQEPVLPVRGP	329	RPKHPIKHQGLPQEVNENLL NLLRFFYQEPVLPVRGP
RPKHPIKHQGLPQEV NENLLRFFV	330	RPKHPIKHQGLPQEVNENLL RFFVYQEPVLPVRGP	331	RPKHPIKHQGLPQEVNENLL NLLRFFVYQEPVLPVRGP
RPKHPIKHQGLPQEV NENLLRFFVA	332	RPKHPIKHQGLPQEVNENLL RFFVAYQEPVLPVRGP	333	RPKHPIKHQGLPQEVNENLL NLLRFFVAYQEPVLPVRGP
	SEQ ID NO:		SEQ ID NO:	
		YQEPVLPVRGPFPI		YQEPVLPVRGPFPI
RP	334	RPYQEPVLPVRGPFPI	335	RPYQEPVLPVRGPFPI
RPK	336	RPKYQEPVLPVRGPFPI	337	RPKYQEPVLPVRGPFPI
RPKH	338	RPKHQEPVLPVRGPFPI	339	RPKHQEPVLPVRGPFPI
RPKHP	340	RPKHQYQEPVLPVRGPFPI	341	RPKHQYQEPVLPVRGPFPI
RPKHPI	342	RPKHPIQEPVLPVRGPFPI	343	RPKHPIQEPVLPVRGPFPI
RPKHPIK	344	RPKHPIKQEPVLPVRGPFPI	345	RPKHPIKQEPVLPVRGPFPI
RPKHPIKH	346	RPKHPIKHQEPVLPVRGPFPI	347	RPKHPIKHQEPVLPVRGPFPI
RPKHPIKHQ	348	RPKHPIKHQYQEPVLPVRGPFPI	349	RPKHPIKHQYQEPVLPVRGPFPI
RPKHPIKHQG	350	RPKHPIKHQGYQEPVLPVRGPFPI	351	RPKHPIKHQGYQEPVLPVRGPFPI
RPKHPIKHQGL	352	RPKHPIKHQGLYQEPVLPVRGPFPI	353	RPKHPIKHQGLYQEPVLPVRGPFPI
RPKHPIKHQGLP	354	RPKHPIKHQGLPYQEPVLPVRGPFPI	355	RPKHPIKHQGLPYQEPVLPVRGPFPI
RPKHPIKHQGLPQ	356	RPKHPIKHQGLPQYQEPVLPVRGPFPI	357	RPKHPIKHQGLPQYQEPVLPVRGPFPI
RPKHPIKHQGLPQE	358	RPKHPIKHQGLPQYQEPVLPVRGPFPI	359	RPKHPIKHQGLPQYQEPVLPVRGPFPI
RPKHPIKHQGLPQEV	360	RPKHPIKHQGLPQEVYQEPVLPVRGPFPI	361	RPKHPIKHQGLPQEVYQEPVLPVRGPFPI
RPKHPIKHQGLPQEV L	362	RPKHPIKHQGLPQEVLYQEPVLPVRGPFPI	363	RPKHPIKHQGLPQEVLYQEPVLPVRGPFPI
RPKHPIKHQGLPQEV N	364	RPKHPIKHQGLPQEVNLYQEPVLPVRGPFPI	365	RPKHPIKHQGLPQEVNLYQEPVLPVRGPFPI
RPKHPIKHQGLPQEV NE	366	RPKHPIKHQGLPQEVNLYQEPVLPVRGPFPI	367	RPKHPIKHQGLPQEVNLYQEPVLPVRGPFPI
RPKHPIKHQGLPQEV NEN	368	RPKHPIKHQGLPQEVNENLYQEPVLPVRGPFPI	369	RPKHPIKHQGLPQEVNENLYQEPVLPVRGPFPI
RPKHPIKHQGLPQEV NENL	370	RPKHPIKHQGLPQEVNENLLYQEPVLPVRGPFPI	371	RPKHPIKHQGLPQEVNENLLYQEPVLPVRGPFPI
RPKHPIKHQGLPQEV NENLL	372	RPKHPIKHQGLPQEVNENLLLYQEPVLPVRGPFPI	373	RPKHPIKHQGLPQEVNENLLLYQEPVLPVRGPFPI
RPKHPIKHQGLPQEV NENLLR	374	RPKHPIKHQGLPQEVNENLLRYQEPVLPVRGPFPI	375	RPKHPIKHQGLPQEVNENLLRYQEPVLPVRGPFPI

Fig. 26g

RPKHPIKHQGLPQEV NENLLRF	376	RPKHPIKHQGLPQEVNENLL RFYQEPVLPVVRGPF	377	RPKHPIKHQGLPQEVNENLL RFYQEPVLPVVRGPF PI
RPKHPIKHQGLPQEV NENLLRFF	378	RPKHPIKHQGLPQEVNENLL RFFYQEPVLPVVRGPF	379	RPKHPIKHQGLPQEVNENLL RFFYQEPVLPVVRGPF FPI
RPKHPIKHQGLPQEV NENLLRFFV	380	RPKHPIKHQGLPQEVNENLL RFFVYQEPVLPVVRGPF	381	RPKHPIKHQGLPQEVNENLL RFFVYQEPVLPVVRGPF PFI
RPKHPIKHQGLPQEV NENLLRFFVA	382	RPKHPIKHQGLPQEVNENLL RFFVAYQEPVLPVVRGPF	383	RPKHPIKHQGLPQEVNENLL RFFVAYQEPVLPVVRGPF GPFPI
	SEQ ID NO:	YQEPVLPVVRGPFPII	SEQ ID NO:	YQEPVLPVVRGPFPIIV
RP	384	RPYQEPVLPVVRGPFPII	385	RPYQEPVLPVVRGPFPII V
RPK	386	RPKYQEPVLPVVRGPFPII	387	RPKYQEPVLPVVRGPFPII IV
RPKH	388	RPKHQEPVLPVVRGPFPII	389	RPKHQEPVLPVVRGPFPII IV
RPKHP	390	RPKHQYQEPVLPVVRGPFPII	391	RPKHQYQEPVLPVVRGPFPII IV
RPKHPI	392	RPKHPIQEPVLPVVRGPFPII	393	RPKHPIQEPVLPVVRGPFPII IV
RPKHPIK	394	RPKHPIKYQEPVLPVVRGPFPII	395	RPKHPIKYQEPVLPVVRGPFPII IV
RPKHPIKH	396	RPKHPIKHQYQEPVLPVVRGPFPII	397	RPKHPIKHQYQEPVLPVVRGPFPII IV
RPKHPIKHQ	398	RPKHPIKHQYQEPVLPVVRGPFPII	399	RPKHPIKHQYQEPVLPVVRGPFPII IV
RPKHPIKHQG	400	RPKHPIKHQGYQEPVLPVVRGPFPII	401	RPKHPIKHQGYQEPVLPVVRGPFPII IV
RPKHPIKHQGL	402	RPKHPIKHQGLYQEPVLPVVRGPFPII	403	RPKHPIKHQGLYQEPVLPVVRGPFPII IV
RPKHPIKHQGLP	404	RPKHPIKHQGLPYQEPVLPVVRGPFPII	405	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
RPKHPIKHQGLPQ	406	RPKHPIKHQGLPYQEPVLPVVRGPFPII	407	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
RPKHPIKHQGLPQE	408	RPKHPIKHQGLPYQEPVLPVVRGPFPII	409	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
RPKHPIKHQGLPQEV	410	RPKHPIKHQGLPYQEPVLPVVRGPFPII	411	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
RPKHPIKHQGLPQEV N	412	RPKHPIKHQGLPYQEPVLPVVRGPFPII	413	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
RPKHPIKHQGLPQEV NEN	414	RPKHPIKHQGLPYQEPVLPVVRGPFPII	415	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
RPKHPIKHQGLPQEV NENL	416	RPKHPIKHQGLPYQEPVLPVVRGPFPII	417	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
RPKHPIKHQGLPQEV NENLL	418	RPKHPIKHQGLPYQEPVLPVVRGPFPII	419	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
	420	RPKHPIKHQGLPYQEPVLPVVRGPFPII	421	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV
	422	RPKHPIKHQGLPYQEPVLPVVRGPFPII	423	RPKHPIKHQGLPYQEPVLPVVRGPFPII IV

Fig. 26h

RPKHPIKHQGLPQEV NENLLR	424	RPKHPIKHQGLPQEVNENLL RYQEPVLGPVRGPFPII	425	RPKHPIKHQGLPQEVNEN NLLRYQEPVLGPVRGPF PIV
RPKHPIKHQGLPQEV NENLLRF	426	RPKHPIKHQGLPQEVNENLL RFYQEPVLGPVRGPFPII	427	RPKHPIKHQGLPQEVNEN NLLRFYQEPVLGPVRGPF PIV
RPKHPIKHQGLPQEV NENLLRFF	428	RPKHPIKHQGLPQEVNENLL RFFYQEPVLGPVRGPFPII	429	RPKHPIKHQGLPQEVNEN NLLRFFYQEPVLGPVRGPF PIV
RPKHPIKHQGLPQEV NENLLRFFV	430	RPKHPIKHQGLPQEVNENLL RFFVYQEPVLGPVRGPFPII	431	RPKHPIKHQGLPQEVNEN NLLRFFVYQEPVLGPVRGPF PIV
RPKHPIKHQGLPQEV NENLLRFFVA	432	RPKHPIKHQGLPQEVNENLL RFFVAYQEPVLGPVRGPFPII	433	RPKHPIKHQGLPQEVNEN NLLRFFVAYQEPVLGPVRGPF PIV

Fig. 26i